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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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(21) International Application Number: PCT/US99/26462 (22) International Filing Date: 10 November 1999 (10.11.1999) (30) Priority Data: 60/107,852 10 November 1998 (10.11.1998) US 60/113,997 28 December 1998 (28.12.1998) US 60/150,604 26 August 1999 (26.08.1999) US 60/157,108 04 October 1999 (04.10.1999) US 60/157,756 05 October 1999 (05.10.1999) US (60) Parent Application or Grant LUDWIG INSTITUTE FOR CANCER RESEARCH [/]; (). HELSINKI UNIVERSITY LICENSING LTD. OY (FI/FI) [/]; (). ERIKSSON, Ulf [/]; (). AASE, Karin [/]; (). PONTEN, Annica [/]; (). LEE, Xuri [/]; (). UUTELA, Marko [/]; (). ALITALO, Kari [/]; (). OESTMAN, Arne [/]; (). HELDIN, Carl-Henrik [/]; (). EVANS, Joseph, D. ; ().		Published
(54) Title: PLATELET-DERIVED GROWTH FACTOR D, DNA CODING THEREFOR, AND USES THEREOF (54) Titre: FACTEUR DE CROISSANCE D D'ORIGINE PLAQUETTAIRE, L'ADN CODANT POUR CELUI-CI, ET SES UTILISATIONS (57) Abstract <p>PDGF-D, a new member of the PDGF/VEGF family of growth factors, is described, as well as the nucleotide sequence encoding it, methods for producing it, antibodies and other antagonists to it, transfected and transformed host cells expressing it, pharmaceutical compositions containing it, and uses thereof in medical and diagnostic applications.</p> (57) Abrégé <p>L'invention concerne PDGF-D, un nouveau membre de la famille PDGF/VEGF des facteurs de croissance, ainsi que la séquence nucléotidique codant pour ce facteur PDGF-D. Cette invention concerne également des procédés de production de ce facteur, des anticorps et d'autres antagonistes de celui-ci, des cellules hôtes transfectées et transformées qui l'expriment, des compositions pharmaceutiques qui le contiennent, et enfin l'utilisation de ce facteur dans des applications médicales et diagnostiques.</p>		

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Description

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PLATELET-DERIVED GROWTH FACTOR D,
DNA CODING THEREFOR, AND USES THEREOF

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This invention relates to growth factors for cells expressing receptors to a novel growth factor that include endothelial cells, connective tissue cells (such as fibroblasts) myofibroblasts and glial cells, and in particular to a novel platelet-derived growth factor/vascular endothelial growth factor-like growth factor, a polynucleotide sequence encoding the factor, and to pharmaceutical and diagnostic compositions and methods utilizing or derived from the factor.

10 BACKGROUND OF THE INVENTION

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In the developing embryo, the primary vascular network is established by *in situ* differentiation of mesodermal cells in a process called vasculogenesis. It is believed that all subsequent processes involving the generation of new vessels in the embryo and neovascularization in adults, are governed by the sprouting or splitting of new capillaries from the pre-existing vasculature in a process called angiogenesis (Pepper et al., Enzyme & Protein, 1996 49 138-162; Breier et al., Dev. Dyn. 1995 204 228-239; Risau, Nature, 1997 386 671-674). Angiogenesis is not only involved in embryonic development and normal tissue growth, repair, and regeneration, but is also involved in the female reproductive cycle, establishment and maintenance of pregnancy, and in repair of wounds and fractures. In addition to angiogenesis which takes place in the normal individual, angiogenic events are involved in a number of pathological processes, notably tumor growth and metastasis, and other conditions in which blood vessel proliferation, especially of the microvascular system, is increased, such as diabetic retinopathy, psoriasis and arthropathies. Inhibition of angiogenesis is useful in preventing or alleviating these pathological processes.

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On the other hand, promotion of angiogenesis is desirable in situations where vascularization is to be established or extended, for example after tissue or organ transplantation, or to stimulate establishment of collateral circulation in tissue infarction or arterial stenosis, such as in coronary heart disease and thromboangitis obliterans.

The angiogenic process is highly complex and involves the maintenance of the endothelial cells in the cell cycle, degradation of the extracellular matrix, migration and invasion of the surrounding tissue and finally, tube formation. The molecular mechanisms underlying the complex angiogenic processes are far from being understood.

Because of the crucial role of angiogenesis in so many physiological and pathological processes, factors involved in the control of angiogenesis have been intensively investigated. A number of growth factors have been shown to be involved in the regulation of angiogenesis; these include fibroblast growth factors (FGFs), platelet-derived growth factor (PDGF), transforming growth factor alpha (TGF α), and hepatocyte growth factor (HGF). See for example Folkman et al., J. Biol. Chem., 1992 267 10931-10934 for a review.

It has been suggested that a particular family of endothelial cell-specific growth factors, the vascular endothelial growth factors (VEGFs), and their corresponding receptors is primarily responsible for stimulation of endothelial cell growth and differentiation, and for certain functions of the differentiated cells. These factors are members of the PDGF family, and appear to act primarily via endothelial receptor tyrosine kinases (RTKs).

Nine different proteins have been identified in the PDGF family, namely two PDGFs (A and B), VEGF and six members that are closely related to VEGF. The six members closely related to VEGF are: VEGF-B, described in International Patent Application PCT/US96/02957 (WO 96/26736) and in U.S. Patents 5,840,693 and

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5,607,918 by Ludwig Institute for Cancer Research and The University of Helsinki; VEGF-C, described in Joukov et al., EMBO J., 1996 15 290-298 and Lee et al., Proc. Natl. Acad. Sci. USA, 1996 93 1988-1992; VEGF-D, described in International Patent Application No. PCT/US97/14696 (WO 98/07832), and Achen et al., Proc. Natl. Acad. Sci. USA, 1998 95 548-553; the placenta growth factor (PlGF), described in Maglione et al., Proc. Natl. Acad. Sci. USA, 1991 88 9267-9271; VEGF2, described in International Patent Application No. PCT/US94/05291 (WO 95/24473) by Human Genome Sciences, Inc; and VEGF3, described in International Patent Application No. PCT/US95/07283 (WO 96/39421) by Human Genome Sciences, Inc. Each VEGF family member has between 30% and 45% amino acid sequence identity with VEGF. The VEGF family members share a VEGF homology domain which contains the six cysteine residues which form the cysteine knot motif. Functional characteristics of the VEGF family include varying degrees of mitogenicity for endothelial cells, induction of vascular permeability and angiogenic and lymphangiogenic properties.

Vascular endothelial growth factor (VEGF) is a homodimeric glycoprotein that has been isolated from several sources. VEGF shows highly specific mitogenic activity for endothelial cells. VEGF has important regulatory functions in the formation of new blood vessels during embryonic vasculogenesis and in angiogenesis during adult life (Carmeliet et al., Nature, 1996 380 435-439; Ferrara et al., Nature, 1996 380 439-442; reviewed in Ferrara and Davis-Smyth, Endocrine Rev., 1997 18 4-25). The significance of the role played by VEGF has been demonstrated in studies showing that inactivation of a single VEGF allele results in embryonic lethality due to failed development of the vasculature (Carmeliet et al., Nature, 1996 380 435-439; Ferrara et al., Nature, 1996 380 439-442). In addition VEGF has strong chemoattractant activity towards monocytes, can induce the plasminogen activator and the plasminogen activator inhibitor in endothelial cells, and can also

5 induce microvascular permeability. Because of the latter
activity, it is sometimes referred to as vascular permeability
10 factor (VPF). The isolation and properties of VEGF have been
reviewed; see Ferrara et al., J. Cellular Biochem., 1991 47 211-
5 218 and Connolly, J. Cellular Biochem., 1991 47 219-223.
Alternative mRNA splicing of a single VEGF gene gives rise to five
15 isoforms of VEGF.

VEGF-B has similar angiogenic and other properties to those
of VEGF, but is distributed and expressed in tissues differently
10 from VEGF. In particular, VEGF-B is very strongly expressed in
heart, and only weakly in lung, whereas the reverse is the case
20 for VEGF. This suggests that VEGF and VEGF-B, despite the fact
that they are co-expressed in many tissues, may have functional
differences.

25 15 VEGF-B was isolated using a yeast co-hybrid interaction trap
screening technique by screening for cellular proteins which might
interact with cellular resinoid acid-binding protein type I
(CRABP-I). Its isolation and characteristics are described in
30 detail in PCT/US96/02957 and in Olofsson et al., Proc. Natl. Acad.
Sci. USA, 1996 93 2576-2581.

35 VEGF-C was isolated from conditioned media of the PC-3
prostate adenocarcinoma cell line (CRL1435) by screening for
ability of the medium to produce tyrosine phosphorylation of the
endothelial cell-specific receptor tyrosine kinase VEGFR-3 (Flt4),
25 using cells transfected to express VEGFR-3. VEGF-C was purified
using affinity chromatography with recombinant VEGFR-3, and was
40 cloned from a PC-3 cDNA library. Its isolation and
characteristics are described in detail in Joukov et al., EMBO J.,
1996 15 290-298.

45 30 VEGF-D was isolated from a human breast cDNA library,
commercially available from Clontech, by screening with an
expressed sequence tag obtained from a human cDNA library
designated "Soares Breast 3NbHBst" as a hybridization probe (Achen

et al., Proc. Natl. Acad. Sci. USA, 1998 95 548-553). Its isolation and characteristics are described in detail in International Patent Application No. PCT/US97/14696 (WO98/07832).

The VEGF-D gene is broadly expressed in the adult human, but is certainly not ubiquitously expressed. VEGF-D is strongly expressed in heart, lung and skeletal muscle. Intermediate levels of VEGF-D are expressed in spleen, ovary, small intestine and colon, and a lower expression occurs in kidney, pancreas, thymus, prostate and testis. No VEGF-D mRNA was detected in RNA from brain, placenta, liver or peripheral blood leukocytes.

PlGF was isolated from a term placenta cDNA library. Its isolation and characteristics are described in detail in Maglione et al., Proc. Natl. Acad. Sci. USA, 1991 88 9267-9271. Presently its biological function is not well understood.

VEGF2 was isolated from a highly tumorigenic, oestrogen-independent human breast cancer cell line. While this molecule is stated to have about 22% homology to PDGF and 30% homology to VEGF, the method of isolation of the gene encoding VEGF2 is unclear, and no characterization of the biological activity is disclosed.

VEGF3 was isolated from a cDNA library derived from colon tissue. VEGF3 is stated to have about 36% identity and 66% similarity to VEGF. The method of isolation of the gene encoding VEGF3 is unclear and no characterization of the biological activity is disclosed.

Similarity between two proteins is determined by comparing the amino acid sequence and conserved amino acid substitutions of one of the proteins to the sequence of the second protein, whereas identity is determined without including the conserved amino acid substitutions.

PDGF/VEGF family members act primarily by binding to receptor tyrosine kinases. Five endothelial cell-specific receptor tyrosine kinases have been identified, namely VEGFR-1 (Flt-1),

5 VEGFR-2 (KDR/Flk-1), VEGFR-3 (Flt4), Tie and Tek/Tie-2. All of
these have the intrinsic tyrosine kinase activity which is
10 necessary for signal transduction. The essential, specific role
in vasculogenesis and angiogenesis of VEGFR-1, VEGFR-2, VEGFR-3,
5 Tie and Tek/Tie-2 has been demonstrated by targeted mutations
inactivating these receptors in mouse embryos.

15 The only receptor tyrosine kinases known to bind VEGFs are
VEGFR-1, VEGFR-2 and VEGFR-3. VEGFR-1 and VEGFR-2 bind VEGF with
high affinity, and VEGFR-1 also binds VEGF-B and PlGF. VEGF-C has
10 been shown to be the ligand for VEGFR-3, and it also activates
VEGFR-2 (Joukov et al., The EMBO Journal, 1996 15 290-298). VEGF-
20 D binds to both VEGFR-2 and VEGFR-3. A ligand for Tek/Tie-2 has
been described in International Patent Application No.
PCT/US95/12935 (WO 96/11269) by Regeneron Pharmaceuticals, Inc.
25 15 The ligand for Tie has not yet been identified.

30 Recently, a novel 130-135 kDa VEGF isoform specific receptor
has been purified and cloned (Soker et al., Cell, 1998 92 735-
745). The VEGF receptor was found to specifically bind the VEGF₁₆₅
isoform via the exon 7 encoded sequence, which shows weak affinity
20 for heparin (Soker et al., Cell, 1998 92 735-745). Surprisingly,
the receptor was shown to be identical to human neuropilin-1 (NP-
1), a receptor involved in early stage neuromorphogenesis. PlGF-2
35 also appears to interact with NP-1 (Migdal et al., J. Biol. Chem.,
1998 273 22272-22278).

25 VEGFR-1, VEGFR-2 and VEGFR-3 are expressed differently by
endothelial cells. Both VEGFR-1 and VEGFR-2 are expressed in
40 blood vessel endothelia (Oelrichs et al., Oncogene, 1992 8 11-18;
Kaipainen et al., J. Exp. Med., 1993 178 2077-2088; Dumont et al.,
Dev. Dyn., 1995 203 80-92; Fong et al., Dev. Dyn., 1996 207 1-10)
45 30 and VEGFR-3 is mostly expressed in the lymphatic endothelium of
adult tissues (Kaipainen et al., Proc. Natl. Acad. Sci. USA, 1995
9 3566-3570). VEGFR-3 is also expressed in the blood vasculature
surrounding tumors.

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Disruption of the VEGFR genes results in aberrant development of the vasculature leading to embryonic lethality around midgestation. Analysis of embryos carrying a completely inactivated VEGFR-1 gene suggests that this receptor is required for functional organization of the endothelium (Fong et al., Nature, 1995 376 66-70). However, deletion of the intracellular tyrosine kinase domain of VEGFR-1 generates viable mice with a normal vasculature (Hiratsuka et al., Proc. Natl. Acad. Sci. USA 1998 95 9349-9354). The reasons underlying these differences remain to be explained but suggest that receptor signalling via the tyrosine kinase is not required for the proper function of VEGFR-1. Analysis of homozygous mice with inactivated alleles of VEGFR-2 suggests that this receptor is required for endothelial cell proliferation, hematopoiesis and vasculogenesis (Shalaby et al., Nature, 1995 376 62-66; Shalaby et al., Cell, 1997 89 981-990). Inactivation of VEGFR-3 results in cardiovascular failure due to abnormal organization of the large vessels (Dumont et al. Science, 1998 282 946-949).

Although VEGFR-1 is mainly expressed in endothelial cells during development, it can also be found in hematopoietic precursor cells during early stages of embryogenesis (Fong et al., Nature, 1995 376 66-70). It is also expressed by most, if not all, vessels in embryos (Breier et al., Dev. Dyn., 1995 204 228-239; Fong et al., Dev. Dyn., 1996 207 1-10). In adults, monocytes and macrophages also express this receptor (Barleon et al., Blood, 1996 87 3336-3343).

The receptor VEGFR-3 is widely expressed on endothelial cells during early embryonic development, but as embryogenesis proceeds, it becomes restricted to venous endothelium and then to the lymphatic endothelium (Kaipainen et al., Cancer Res., 1994 54 6571-6577; Kaipainen et al., Proc. Natl. Acad. Sci. USA, 1995 92 3566-3570). VEGFR-3 continues to be expressed on lymphatic endothelial cells in adults. This receptor is essential for

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vascular development during embryogenesis. Targeted inactivation of both copies of the VEGFR-3 gene in mice resulted in defective blood vessel formation characterized by abnormally organized large vessels with defective lumens, leading to fluid accumulation in the pericardial cavity and cardiovascular failure at post-coital day 9.5. On the basis of these findings it has been proposed that VEGFR-3 is required for the maturation of primary vascular networks into larger blood vessels. However, the role of VEGFR-3 in the development of the lymphatic vasculature could not be studied in these mice because the embryos died before the lymphatic system emerged. Nevertheless it is assumed that VEGFR-3 plays a role in development of the lymphatic vasculature and lymphangiogenesis given its specific expression in lymphatic endothelial cells during embryogenesis and adult life. This is supported by the finding that ectopic expression of VEGF-C, a ligand for VEGFR-3, in the skin of transgenic mice, resulted in lymphatic endothelial cell proliferation and vessel enlargement in the dermis. Furthermore this suggests that VEGF-C may have a primary function in lymphatic endothelium, and a secondary function in angiogenesis and permeability regulation which is shared with VEGF (Joukov et al., EMBO J., 1996 15 290-298).

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Some inhibitors of the VEGF/VEGF-receptor system have been shown to prevent tumor growth via an anti-angiogenic mechanism; see Kim et al., Nature, 1993 362 841-844 and Saleh et al., Cancer Res., 1996 56 393-401.

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As mentioned above, the VEGF family of growth factors are members of the PDGF family. PDGF plays a important role in the growth and/or motility of connective tissue cells, fibroblasts, myofibroblasts and glial cells (Heldin et al., "Structure of platelet-derived growth factor: Implications for functional properties", Growth Factor, 1993 8 245-252). In adults, PDGF stimulates wound healing (Robson et al., Lancet, 1992 339 23-25). Structurally, PDGF isoforms are disulfide-bonded dimers of

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homologous A- and B-polypeptide chains, arranged as homodimers (PDGF-AA and PDGF-BB) or a heterodimer (PDGF-AB).

PDGF isoforms exert their effects on target cells by binding to two structurally related receptor tyrosine kinases (RTKs). The alpha-receptor binds both the A- and B-chains of PDGF, whereas the beta-receptor binds only the B-chain. These two receptors are expressed by many *in vitro* grown cell lines, and are mainly expressed by mesenchymal cells *in vivo*. The PDGFs regulate cell proliferation, cell survival and chemotaxis of many cell types *in vitro* (reviewed in Heldin et al., *Biochim Biophys Acta.*, 1998 1378 F79-113). *In vivo*, they exert their effects in a paracrine mode since they often are expressed in epithelial (PDGF-A) or endothelial cells (PDGF-B) in close apposition to the PDGFR expressing mesenchyme. In tumor cells and in cell lines grown *in vitro*, coexpression of the PDGFs and the receptors generate autocrine loops which are important for cellular transformation (Betsholtz et al., *Cell*, 1984 39 447-57; Keating et al., *J. R. Coll Surg Edinb.*, 1990 35 172-4). Overexpression of the PDGFs have been observed in several pathological conditions, including malignancies, arteriosclerosis, and fibroproliferative diseases (reviewed in Heldin et al., *The Molecular and Cellular Biology of Wound Repair*, New York: Plenum Press, 1996, 249-273).

The importance of the PDGFs as regulators of cell proliferation and survival are well illustrated by recent gene targeting studies in mice that have shown distinct physiological roles for the PDGFs and their receptors despite the overlapping ligand specificities of the PDGFRs. Homozygous null mutations for either of the two PDGF ligands or the receptors are lethal. Approximately 50% of the homozygous PDGF-A deficient mice have an early lethal phenotype, while the surviving animals have a complex postnatal phenotype with lung emphysema due to improper alveolar septum formation because of a lack of alveolar myofibroblasts (Boström et al., *Cell*, 1996 85 863-873). The PDGF-A deficient

5 mice also have a dermal phenotype characterized by thin dermis,
misshapen hair follicles and thin hair (Karlsson et al.,
10 Development, 1999 126 2611-2). PDGF-A is also required for normal
development of oligodendrocytes and subsequent myelination of the
5 central nervous system (Fruttiger et al., Development, 1999 126
457-67). The phenotype of PDGFR-alpha deficient mice is more
15 severe with early embryonic death at E10, incomplete cephalic
closure, impaired neural crest development, cardiovascular
defects, skeletal defects, and odemas [Soriano et al.,
10 Development, 1997 124 2691-70). The PDGF-B and PDGFR-beta
deficient mice develop similar phenotypes that are characterized
by renal, hematological and cardiovascular abnormalities (Levéen
et al., Genes Dev., 1994 8 1875-1887; Soriano et al., Genes Dev.,
1994 8 1888-96; Lindahl et al., Science, 1997 277 242-5; Lindahl,
25 Development, 1998 125 3313-2), where the renal and cardiovascular
defects, at least in part, are due to the lack of proper
recruitment of mural cells (vascular smooth muscle cells,
pericytes or mesangial cells) to blood vessels (Levéen et al.,
30 Genes Dev., 1994 8 1875-1887; Lindahl et al., Science, 1997 277
242-5; Lindahl et al., Development, 1998 125 3313-2).

SUMMARY OF THE INVENTION

35 The invention generally provides an isolated novel growth
25 factor which has the ability to stimulate and/or enhance
proliferation or differentiation and/or growth and/or motility of
40 cells expressing a PDGF-D receptor including, but not limited to,
endothelial cells, connective tissue cells, myofibroblasts and
glial cells, an isolated polynucleotide sequence encoding the
45 novel growth factor, and compositions useful for diagnostic and/or
30 therapeutic applications.

According to one aspect, the invention provides an isolated
and purified nucleic acid molecule which comprises a

polynucleotide sequence having at least 85% identity, more preferably at least 90%, and most preferably at least 95% identity to at least nucleotides 1 to 600 of the sequence set out in Figure 3 (SEQ ID NO:3), at least nucleotides 1 to 966 of the sequence set out in Figure 5 (SEQ ID NO:5), at least nucleotides 176 to 1288 of the sequence set out in Figure 7 (SEQ ID NO:7) or at least nucleotides 938 to 1288 set out in Figure 7 (SEQ ID NO:7). The sequence of at least nucleotides 1 to 600 of the sequence set out in Figure 3 or at least nucleotides 1 to 966 of the sequence set out in Figure 5 encodes a 5'-truncated polypeptide, designated PDGF-D (formally designated "VEGF-G"), while at least nucleotides 173 to 1288 of the sequence set out in Figure 7 (SEQ ID NO:7) encodes a full-length PDGF-D. PDGF-D is structurally homologous to PDGF-A, PDGF-B, VEGF, VEGF-B, VEGF-C and VEGF-D. The sequence of at least nucleotides 938 to 1288 set out in Figure 7 (SEQ ID NO:7) encodes a portion of the PDGF/VEGF homology domain, which is the bioactive fragment of PDGF-D. This bioactive fragment would also be encoded by the sequence of at least nucleotides 1 to 600 of the sequence set out in Figure 3 or at least nucleotides 1 to 966 of the sequence set out in Figure 5. In a preferred embodiment, the nucleic acid molecule is a cDNA which comprises at least nucleotides 1 to 600 of the sequence set out in Figure 3 (SEQ ID NO:3), at least nucleotides 1 to 966 of the sequence set out in Figure 5 (SEQ ID NO:5), at least nucleotides 173 to 1288 of the sequence set out in Figure 7 (SEQ ID NO:7) or at least nucleotides 938 to 1288 set out in Figure 7 (SEQ ID NO:7). This aspect of the invention also encompasses DNA molecules having a sequence such that they hybridize under stringent conditions with at least nucleotides 1 to 600 of the sequence set out in Figure 3 (SEQ ID NO:3), at least nucleotides 1 to 966 of the sequence set out in Figure 5 (SEQ ID NO:5), at least nucleotides 173 to 1288 of the sequence set out in Figure 7 (SEQ ID NO:7) or at least

5 nucleotides 938 to 1288 set out in Figure 7 (SEQ ID NO:7) or fragments thereof.

10 According to a second aspect, the polypeptide of the invention has the ability to stimulate and/or enhance
5 proliferation and/or differentiation and/or growth and/or motility of cells expressing a PDGF-C receptor including, but not limited to, endothelial cells, connective tissue cells, myofibroblasts
15 and glial cells and comprises a sequence of amino acids corresponding to the amino acid sequence set out in Figure 4 (SEQ ID NO:4) or Figure 6 (SEQ ID NO:6), or Figure 8 (SEQ ID NO:8),
10 or a fragment or analog thereof which has the ability to stimulate endothelial cell proliferation, differentiation, migration and/or survival of and/or growth and/or motility of connective tissue cells (such as fibroblasts), myofibroblasts and glial cells.
20 Preferably the polypeptides have at least 85% identity, more preferably at least 90%, and most preferably at least 95% identity to the amino acid sequence of in Figure 4 (SEQ ID NO:4) or Figure 6 (SEQ ID NO:6) or Figure 8 (SEQ ID NO:8), or a fragment or analog thereof having the biological activity of PDGF-D. A preferred
30 fragment is a truncated form of PDGF-D comprising a portion of the PDGF/VEGF homology domain (PVHD) of PDGF-D. The portion of the PVHD is from residues 255-371 of Figure 8 where the putative proteolytic processing site RKSK starts at amino acid residue 255 (SEQ ID NO:8). However, the PVHD extends toward the N terminus
35 up to residue 235 of Figure 8 (SEQ ID NO:8). Herein the PVHD is defined as truncated PDGF-D. The truncated PDGF-D is the putative activated form of PDGF-D.
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As used in this application, percent sequence identity is determined by using the alignment tool of "MEGALIGN" from the
45 Lasergene package (DNASTAR, Ltd. Abacus House, Manor Road, West Ealing, London W130AS United Kingdom). The MEGALIGN is based on the J. Hein method (Methods in Enzymology, 1990 183 626-645). The PAM 250 residue weight table is used with a gap penalty of eleven
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5 and a gap length penalty of three and a K-tuple value of two in
the pairwise alignments. The alignment is then refined manually,
10 and the number of identities are estimated in the regions
available for a comparison.

5 Preferably the polypeptide or the encoded polypeptide from
a polynucleotide has the ability to stimulate one or more of
15 proliferation, differentiation, motility, survival or vascular
permeability of cells expressing a PDGF-D receptor including, but
not limited to, vascular endothelial cells, lymphatic endothelial
10 cells, connective tissue cells (such as fibroblasts),
myofibroblasts and glial cells. Preferably the polypeptide or the
20 encoded polypeptide from a polynucleotide has the ability to
stimulate wound healing. PDGF-D can also have antagonistic
effects on cells, but are included in the biological activities
25 15 of PDGF-D. These abilities are referred to hereinafter as
"biological activities of PDGF-D" and can be readily tested by
methods known in the art.

30 As used herein, the term "PDGF-D" collectively refers to the
polypeptides of Figure 4 (SEQ ID NO:4), Figure 6 (SEQ ID NO:6) or
20 Figure 8 (SEQ ID NO:8), and fragments or analogs thereof which
have the biological activity of PDGF-D as defined above, and to
a polynucleotide which can code for PDGF-D, or a fragment or
35 analog thereof having the biological activity of PDGF-D. The
polynucleotide can be naked and/or in a vector or liposome.

25 In another preferred aspect, the invention provides a
polypeptide possessing an amino acid sequence:

40 PXCLLVXRCGGNCXC (SEQ ID NO:25)

which is unique to PDGF-D and differs from the other members of
the PDGF/VEGF family of growth factors because of the insertion
45 30 of the three amino acid residues (NCG) between the third and
fourth cysteines (see Figure 9 - SEQ ID NOs:10-18).

Polypeptides comprising conservative substitutions,
insertions, or deletions, but which still retain the biological

activity of PDGF-D are clearly to be understood to be within the scope of the invention. Persons skilled in the art will be well aware of methods which can readily be used to generate such polypeptides, for example the use of site-directed mutagenesis, or specific enzymatic cleavage and ligation. The skilled person will also be aware that peptidomimetic compounds or compounds in which one or more amino acid residues are replaced by a non-naturally occurring amino acid or an amino acid analog may retain the required aspects of the biological activity of PDGF-D. Such compounds can readily be made and tested for their ability to show the biological activity of PDGF-D by routine activity assay procedures such as the fibroblast proliferation assay and are also within the scope of the invention.

In addition, possible variant forms of the PDGF-D polypeptide which may result from alternative splicing, as are known to occur with VEGF and VEGF-B, and naturally-occurring allelic variants of the nucleic acid sequence encoding PDGF-D are encompassed within the scope of the invention. Allelic variants are well known in the art, and represent alternative forms or a nucleic acid sequence which comprise substitution, deletion or addition of one or more nucleotides, but which do not result in any substantial functional alteration of the encoded polypeptide.

Such variant forms of PDGF-D can be prepared by targeting non-essential regions of the PDGF-D polypeptide for modification. These non-essential regions are expected to fall outside the strongly-conserved regions indicated in Figure 9 (SEQ ID NOs:10-18). In particular, the growth factors of the PDGF family, including PDGF-D, are dimeric. PDGF-D differs slightly from VEGF, VEGF-B, VEGF-C, VEGF-D, PlGF, PDGF-A and PDGF-B because it shows complete conservation of only seven of the eight cysteine residues in the PVHD (Olofsson et al., Proc. Natl. Acad. Sci. USA, 1996 93 2576-2581; Joukov et al., EMBO J., 1996 15 290-298). These cysteines are thought to be involved in intra- and inter-molecular

disulfide bonding. Loops 1, 2 and 3 of each subunit, which are formed by intra-molecular disulfide bonding, are involved in binding to the receptors for the PDGF/VEGF family of growth factors (Andersson et al., Growth Factors, 1995 12 159-164).

Persons skilled in the art thus are well aware that these cysteine residues should be preserved in any proposed variant form, and that the active sites present in loops 1, 2 and 3 also should be preserved. However, other regions of the molecule can be expected to be of lesser importance for biological function, and therefore offer suitable targets for modification. Modified polypeptides can readily be tested for their ability to show the biological activity of PDGF-D by routine activity assay procedures such as the fibroblast proliferation assay.

It is contemplated that some modified PDGF-D polypeptides will have the ability to bind to PDGF-D receptors on cells including, but not limited to, endothelial cells, connective tissue cells, myofibroblasts and/or glial cells, but will be unable to stimulate cell proliferation, differentiation, migration, motility or survival or to induce vascular proliferation, connective tissue development or wound healing. These modified polypeptides are expected to be able to act as competitive or non-competitive inhibitors of the PDGF-D polypeptides and growth factors of the PDGF/VEGF family, and to be useful in situations where prevention or reduction of the PDGF-D polypeptide or PDGF/VEGF family growth factor action is desirable. Thus such receptor-binding but non-mitogenic, non-differentiation inducing, non-migration inducing, non-motility inducing, non-survival promoting, non-connective tissue promoting, non-wound healing or non-vascular proliferation inducing variants of the PDGF-D polypeptide are also within the scope of the invention, and are referred to herein as "receptor-binding but otherwise inactive variants". Because PDGF-D forms a dimer in order to activate its only known receptor, it is contemplated that

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one monomer comprises the receptor-binding but otherwise inactive variant modified PDGF-D polypeptide and a second monomer comprises a wild-type PDGF-D or a wild-type growth factor of the PDGF/VEGF family. These dimers can bind to its corresponding receptor but cannot induce downstream signaling.

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It is also contemplated that there are other modified PDGF-D polypeptides that can prevent binding of a wild-type PDGF-D or a wild-type growth factor of the PDGF/VEGF family to its corresponding receptor on cells including, but not limited to, endothelial cells, connective tissue cells (such as fibroblasts), myofibroblasts and/or glial cells. Thus these dimers will be unable to stimulate endothelial cell proliferation, differentiation, migration, survival, or induce vascular permeability, and/or stimulate proliferation and/or differentiation and/or motility of connective tissue cells, myofibroblasts or glial cells. These modified polypeptides are expected to be able to act as competitive or non-competitive inhibitors of the PDGF-D growth factor or a growth factor of the PDGF/VEGF family, and to be useful in situations where prevention or reduction of the PDGF-D growth factor or PDGF/VEGF family growth factor action is desirable. Such situations include the tissue remodeling that takes place during invasion of tumor cells into a normal cell population by primary or metastatic tumor formation. Thus such the PDGF-D or PDGF/VEGF family growth factor-binding but non-mitogenic, non-differentiation inducing, non-migration inducing, non-motility inducing, non-survival promoting, non-connective tissue promoting, non-wound healing or non-vascular proliferation inducing variants of the PDGF-D growth factor are also within the scope of the invention, and are referred to herein as "the PDGF-D growth factor-dimer forming but otherwise inactive or interfering variants".

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An example of a PDGF-D growth factor-dimer forming but otherwise inactive or interfering variant is where the PDGF-D has

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5 a mutation which prevents cleavage of CUB domain from the protein.
It is further contemplated that a PDGF-D growth factor-dimer
10 forming but otherwise inactive or interfering variant could be
made to comprise a monomer, preferably an activated monomer, of
5 VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-C, PDGF-A, PDGF-B, PDGF-C,
PDGF-D or PlGF linked to a CUB domain that has a mutation which
15 prevents cleavage of CUB domain from the protein. Dimers formed
with the above mentioned PDGF-D growth factor-dimer forming but
otherwise inactive or interfering variants and the monomers linked
10 to the mutant CUB domain would be unable to bind to their
corresponding receptors.
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A variation on this contemplation would be to insert a
proteolytic site between an activated monomer of VEGF, VEGF-B,
25 VEGF-C, VEGF-D, PDGF-C, PDGF-A, PDGF-B, PDGF-C, PDGF-D or PlGF and
15 the mutant CUB domain linkage which is dimerized to an activated
monomer of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-A, PDGF-B, PDGF-C,
PDGF-D or PlGF. An addition of the specific protease(s) for this
30 proteolytic site would cleave the CUB domain and thereby release
an activated dimer that can then bind to its corresponding
20 receptor. In this way, a controlled release of an activated dimer
is made possible.

According to a third aspect, the invention provides a
35 purified and isolated nucleic acid encoding a polypeptide or
polypeptide fragment of the invention as defined above. The
25 nucleic acid may be DNA, genomic DNA, cDNA or RNA, and may be
single-stranded or double stranded. The nucleic acid may be
40 isolated from a cell or tissue source, or of recombinant or
synthetic origin. Because of the degeneracy of the genetic code,
the person skilled in the art will appreciate that many such
30 coding sequences are possible, where each sequence encodes the
amino acid sequence shown in Figure 4 (SEQ ID NO:4), Figure 6 (SEQ
ID NO:6) or Figure 8 (SEQ ID NO:8), a bioactive fragment or analog
thereof, a receptor-binding but otherwise inactive or partially
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5 inactive variant thereof or a PDGF-D dimer-forming but otherwise inactive or interfering variants thereof.

10 A fourth aspect of the invention provides vectors comprising the cDNA of the invention or a nucleic acid molecule according to
5 the third aspect of the invention, and host cells transformed or transfected with nucleic acids molecules or vectors of the invention. These may be eukaryotic or prokaryotic in origin.
15 These cells are particularly suitable for expression of the polypeptide of the invention, and include insect cells such as Sf9 cells, obtainable from the American Type Culture Collection
10 (ATCC SRL-171), transformed with a baculovirus vector, and the human embryo kidney cell line 293-EBNA transfected by a suitable expression plasmid. Preferred vectors of the invention are
20 expression vectors in which a nucleic acid according to the invention is operatively connected to one or more appropriate
25 promoters and/or other control sequences, such that appropriate host cells transformed or transfected with the vectors are capable of expressing the polypeptide of the invention. Other preferred
30 vectors are those suitable for transfection of mammalian cells, or for gene therapy, such as adenoviral-, vaccinia- or retroviral-based vectors or liposomes. A variety of such vectors is known
in the art.

35 The invention also provides a method of making a vector capable of expressing a polypeptide encoded by a nucleic acid
25 molecule according to the invention, comprising the steps of operatively connecting the nucleic acid molecule to one or more appropriate promoters and/or other control sequences, as described
40 above.

45 The invention further provides a method of making a polypeptide according to the invention, comprising the steps of
30 expressing a nucleic acid or vector of the invention in a host cell, and isolating the polypeptide from the host cell or from the host cell's growth medium.

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In yet a further aspect, the invention provides an antibody specifically reactive with a polypeptide of the invention or a fragment of the polypeptide. This aspect of the invention includes antibodies specific for the variant forms, immunoreactive fragments, analogs and recombinants of PDGF-D. Such antibodies are useful as inhibitors or agonists of PDGF-D and as diagnostic agents for detecting and quantifying PDGF-D. Polyclonal or monoclonal antibodies may be used. Monoclonal and polyclonal antibodies can be raised against polypeptides of the invention or fragment or analog thereof using standard methods in the art. In addition the polypeptide can be linked to an epitope tag, such as the FLAG[®] octapeptide (Sigma, St. Louis, MO), to assist in affinity purification. For some purposes, for example where a monoclonal antibody is to be used to inhibit effects of PDGF-D in a clinical situation, it may be desirable to use humanized or chimeric monoclonal antibodies. Such antibodies may be further modified by addition of cytotoxic or cytostatic drug(s). Methods for producing these, including recombinant DNA methods, are also well known in the art.

This aspect of the invention also includes an antibody which recognizes PDGF-D and is suitably labeled.

Polypeptides or antibodies according to the invention may be labeled with a detectable label, and utilized for diagnostic purposes. Similarly, the thus-labeled polypeptide of the invention may be used to identify its corresponding receptor *in situ*. The polypeptide or antibody may be covalently or non-covalently coupled to a suitable supermagnetic, paramagnetic, electron dense, ecogenic or radioactive agent for imaging. For use in diagnostic assays, radioactive or non-radioactive labels may be used. Examples of radioactive labels include a radioactive atom or group, such as ¹²⁵I or ³²P. Examples of non-radioactive labels include enzymatic labels, such as horseradish peroxidase or fluorimetric labels, such as fluorescein-5-isothiocyanate

(FITC). Labeling may be direct or indirect, covalent or non-covalent.

Clinical applications of the invention include diagnostic applications, acceleration of angiogenesis in tissue or organ transplantation, or stimulation of wound healing, or connective tissue development, or to establish collateral circulation in tissue infarction or arterial stenosis, such as coronary artery disease, and inhibition of angiogenesis in the treatment of cancer or of diabetic retinopathy and inhibition of tissue remodeling that takes place during invasion of tumor cells into a normal cell population by primary or metastatic tumor formation. Quantitation of PDGF-D in cancer biopsy specimens may be useful as an indicator of future metastatic risk.

PDGF-D may also be relevant to a variety of lung conditions. PDGF-D assays could be used in the diagnosis of various lung disorders. PDGF-D could also be used in the treatment of lung disorders to improve blood circulation in the lung and/or gaseous exchange between the lungs and the blood stream. Similarly, PDGF-D could be used to improve blood circulation to the heart and O₂ gas permeability in cases of cardiac insufficiency. In a like manner, PDGF-D could be used to improve blood flow and gaseous exchange in chronic obstructive airway diseases.

Thus the invention provides a method of stimulation of angiogenesis, lymphangiogenesis, neovascularization, connective tissue development and/or wound healing in a mammal in need of such treatment, comprising the step of administering an effective dose of PDGF-D, or a fragment or an analog thereof which has the biological activity of PDGF-D to the mammal. Optionally the PDGF-D, or fragment or analog thereof may be administered together with, or in conjunction with, one or more of VEGF, VEGF-B, VEGF-C, VEGF-D, PlGF, PDGF-A, PDGF-B, PDGF-C, FGF and/or heparin.

Conversely, PDGF-D antagonists (e.g. antibodies and/or competitive or noncompetitive inhibitors of binding of PDGF-D in

5 both dimer formation and receptor binding) could be used to treat
conditions, such as congestive heart failure, involving
10 accumulation of fluid in, for example, the lung resulting from
increases in vascular permeability, by exerting an offsetting
5 effect on vascular permeability in order to counteract the fluid
accumulation. Administrations of PDGF-D could be used to treat
15 malabsorptive syndromes in the intestinal tract, liver or kidneys
as a result of its blood circulation increasing and vascular
permeability increasing activities.

10 Thus, the invention provides a method of inhibiting
angiogenesis, lymphangiogenesis, neovascularization, connective
20 tissue development and/or wound healing in a mammal in need of
such treatment, comprising the step of administering an effective
amount of an antagonist of PDGF-D to the mammal. The antagonist
25 15 may be any agent that prevents the action of PDGF-D, either by
preventing the binding of PDGF-D to its corresponding receptor on
the target cell, or by preventing activation of the receptor, such
as using receptor-binding PDGF-D variants. Suitable antagonists
30 include, but are not limited to, antibodies directed against PDGF-
20 D; competitive or non-competitive inhibitors of binding of PDGF-D
to the PDGF-D receptor(s), such as the receptor-binding or PDGF-D
dimer-forming but non-mitogenic PDGF-D variants referred to above;
35 and anti-sense nucleotide sequences as described below.

A method is provided for determining agents that bind to an
25 activated truncated form of PDGF-D. The method comprises
contacting an activated truncated form of PDGF-D with a test agent
40 and monitoring binding by any suitable means. Agents can include
both compounds and other proteins.

The invention provides a screening system for discovering
45 30 agents that bind an activated truncated form of PDGF-D. The
screening system comprises preparing an activated truncated form
of PDGF-D, exposing the activated truncated form of PDGF-D to a
test agent, and quantifying the binding of said agent to the

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activated truncated form of PDGF-D by any suitable means. This screening system can also be used to identify agents which inhibit the proteolytic cleavage of the full length PDGF-D protein and thereby prevent the release of the activated truncated form of PDGF-D. For this use, the full length PDGF-D must be prepared.

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Use of this screen system provides a means to determine compounds that may alter the biological function of PDGF-D. This screening method may be adapted to large-scale, automated procedures such as a PANDEX® (Baxter-Dade Diagnostics) system, allowing for efficient high-volume screening of potential therapeutic agents.

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For this screening system, an activated truncated form of PDGF-D or full length PDGF-D is prepared as described herein, preferably using recombinant DNA technology. A test agent, e.g. a compound or protein, is introduced into a reaction vessel containing the activated truncated form of or full length PDGF-D. Binding of the test agent to the activated truncated form of or full length PDGF-D is determined by any suitable means which include, but is not limited to, radioactively- or chemically-labeling the test agent. Binding of the activated truncated form of or full length PDGF-D may also be carried out by a method disclosed in U.S. Patent 5,585,277, which is incorporated by reference. In this method, binding of the test agent to the activated truncated form of or full length PDGF-D is assessed by monitoring the ratio of folded protein to unfolded protein. Examples of this monitoring can include, but are not limited to, monitoring the sensitivity of the activated truncated form of or full length PDGF-D to a protease, or amenability to binding of the protein by a specific antibody against the folded state of the protein.

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Those of skill in the art will recognize that IC_{50} values are dependent on the selectivity of the agent tested. For example, an agent with an IC_{50} which is less than 10 nM is generally

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5 considered an excellent candidate for drug therapy. However, an
agent which has a lower affinity, but is selective for a
10 particular target, may be an even better candidate. Those skilled
in the art will recognize that any information regarding the
5 binding potential, inhibitory activity or selectivity of a
particular agent is useful toward the development of
15 pharmaceutical products.

Where PDGF-D or a PDGF-D antagonist is to be used for
therapeutic purposes, the dose(s) and route of administration will
10 depend upon the nature of the patient and condition to be treated,
and will be at the discretion of the attending physician or
20 veterinarian. Suitable routes include oral, subcutaneous,
intramuscular, intraperitoneal or intravenous injection,
parenteral, topical application, implants etc. Topical
25 application of PDGF-D may be used in a manner analogous to VEGF.
Where used for wound healing or other use in which enhanced
angiogenesis is advantageous, an effective amount of the truncated
active form of PDGF-D is administered to an organism in need
30 thereof in a dose between about 0.1 and 1000 µg/kg body weight.

20 The PDGF-D or a PDGF-D antagonist may be employed in
combination with a suitable pharmaceutical carrier. The resulting
compositions comprise a therapeutically effective amount of PDGF-D
35 or a PDGF-D antagonist, and a pharmaceutically acceptable non-
toxic salt thereof, and a pharmaceutically acceptable solid or
25 liquid carrier or adjuvant. Examples of such a carrier or
adjuvant include, but are not limited to, saline, buffered saline,
40 Ringer's solution, mineral oil, talc, corn starch, gelatin,
lactose, sucrose, microcrystalline cellulose, kaolin, mannitol,
dicalcium phosphate, sodium chloride, alginic acid, dextrose,
45 30 water, glycerol, ethanol, thickeners, stabilizers, suspending
agents and combinations thereof. Such compositions may be in the
form of solutions, suspensions, tablets, capsules, creams, salves,
elixirs, syrups, wafers, ointments or other conventional forms.

5 The formulation to suit the mode of administration. Compositions
which comprise PDGF-D may optionally further comprise one or more
10 of PDGF-A, PDGF-B, PDGF-C, VEGF, VEGF-B, VEGF-C, VEGF-D, PlGF
and/or heparin. Compositions comprising PDGF-D will contain from
5 about 0.1% to 90% by weight of the active compound(s), and most
generally from about 10% to 30%.

15 For intramuscular preparations, a sterile formulation,
preferably a suitable soluble salt form of the truncated active
form of PDGF-D, such as hydrochloride salt, can be dissolved and
10 administered in a pharmaceutical diluent such as pyrogen-free
water (distilled), physiological saline or 5% glucose solution.
20 A suitable insoluble form of the compound may be prepared and
administered as a suspension in an aqueous base or a
pharmaceutically acceptable oil base, e.g. an ester of a long
25 chain fatty acid such as ethyl oleate.

According to yet a further aspect, the invention provides
diagnostic/prognostic devices typically in the form of test kits.
For example, in one embodiment of the invention there is provided
30 a diagnostic/prognostic test kit comprising an antibody to PDGF-D
and a means for detecting, and more preferably evaluating, binding
20 between the antibody and PDGF-D. In one preferred embodiment of
the diagnostic/prognostic device according to the invention, a
35 second antibody (the secondary antibody) directed against
antibodies of the same isotype and animal source of the antibody
25 directed against PDGF-D (the primary antibody) is provided. The
secondary antibody is coupled directly or indirectly to a
40 detectable label, and then either an unlabeled primary antibody
or PDGF-D is substrate-bound so that the PDGF-D/primary antibody
interaction can be established by determining the amount of label
45 bound to the substrate following binding between the primary
antibody and PDGF-D and the subsequent binding of the labeled
secondary antibody to the primary antibody. In a particularly
preferred embodiment of the invention, the diagnostic/prognostic

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device may be provided as a conventional enzyme-linked immunosorbent assay (ELISA) kit.

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In another alternative embodiment, a diagnostic/prognostic device may comprise polymerase chain reaction means for establishing sequence differences of a PDGF-D of a test individual and comparing this sequence structure with that disclosed in this application in order to detect any abnormalities, with a view to establishing whether any aberrations in PDGF-D expression are related to a given disease condition.

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In addition, a diagnostic/prognostic device may comprise a restriction length polymorphism (RFLP) generating means utilizing restriction enzymes and genomic DNA from a test individual to generate a pattern of DNA bands on a gel and comparing this pattern with that disclosed in this application in order to detect any abnormalities, with a view to establishing whether any aberrations in PDGF-D expression are related to a given disease condition.

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In accordance with a further aspect, the invention relates to a method of detecting aberrations in PDGF-D gene structure in a test subject which may be associated with a disease condition in the test subject. This method comprises providing a DNA sample from said test subject; contacting the DNA sample with a set of primers specific to PDGF-D DNA operatively coupled to a polymerase and selectively amplifying PDGF-D DNA from the sample by polymerase chain reaction, and comparing the nucleotide sequence of the amplified PDGF-D DNA from the sample with the nucleotide sequences shown in Figure 3 (SEQ ID NO:3), Figure 5 (SEQ ID NO:5) or Figure 7 (SEQ ID NO:7). The invention also includes the provision of a test kit comprising a pair of primers specific to PDGF-D DNA operatively coupled to a polymerase, whereby said polymerase is enabled to selectively amplify PDGF-D DNA from a DNA sample.

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The invention also provides a method of detecting PDGF-D in a biological sample, comprising the step of contacting the sample with a reagent capable of binding PDGF-D, and detecting the binding. Preferably the reagent capable of binding PDGF-D is an antibody directed against PDGF-D, particularly a monoclonal antibody. In a preferred embodiment the binding and/or extent of binding is detected by means of a detectable label; suitable labels are discussed above.

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In another aspect, the invention relates to a protein dimer comprising the PDGF-D polypeptide, particularly a disulfide-linked dimer. The protein dimers of the invention include both homodimers of PDGF-D polypeptide and heterodimers of PDGF-D and VEGF, VEGF-B, VEGF-C, VEGF-D, PlGF, PDGF-A, PDGF-B or PDGF-C.

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According to a yet further aspect of the invention there is provided a method for isolation of PDGF-D comprising the step of exposing a cell which expresses PDGF-D to heparin to facilitate release of PDGF-D from the cell, and purifying the thus-released PDGF-D.

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Another aspect of the invention involves providing a vector comprising an anti-sense nucleotide sequence which is complementary to at least a part of a DNA sequence which encodes PDGF-D or a fragment or analog thereof that has the biological activity of PDGF-D. In addition the anti-sense nucleotide sequence can be to the promoter region of the PDGF-D gene or other non-coding region of the gene which may be used to inhibit, or at least mitigate, PDGF-D expression.

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According to a yet further aspect of the invention such a vector comprising an anti-sense sequence may be used to inhibit, or at least mitigate, PDGF-D expression. The use of a vector of this type to inhibit PDGF-D expression is favored in instances where PDGF-D expression is associated with a disease, for example where tumors produce PDGF-D in order to provide for angiogenesis, or tissue remodeling that takes place during invasion of tumor

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cells into a normal cell population by primary or metastatic tumor formation. Transformation of such tumor cells with a vector containing an anti-sense nucleotide sequence would suppress or retard angiogenesis, and so would inhibit or retard growth of the tumor or tissue remodeling.

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Another aspect of the invention relates to the discovery that the full length PDGF-D protein is likely to be a latent growth factor that needs to be activated by proteolytic processing to release an active PDGF/VEGF homology domain. A putative proteolytic site is found in residues 255-258 in the full length protein, residues -RKSK- (SEQ ID NO:9). This is a dibasic motif. The -RKSK- (SEQ ID NO:9) putative proteolytic site is also found in PDGF-A, PDGF-B, VEGF-C and VEGF-D. In these four proteins, the putative proteolytic site is also found just before the minimal domain for the PDGF/VEGF homology domain. Together these facts indicate that this is the proteolytic site.

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Preferred proteases include, but are not limited, to plasmin, Factor X and enterokinase. The N-terminal CUB domain may function as an inhibitory domain which might be used to keep PDGF-D in a latent form in some extracellular compartment and which is removed by limited proteolysis when PDGF-D is needed.

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According to this aspect of the invention, a method is provided for producing an activated truncated form of PDGF-D or for regulating receptor-binding specificity of PDGF-D. These methods comprise the steps of expressing an expression vector comprising a polynucleotide encoding a polypeptide having the biological activity of PDGF-D and supplying a proteolytic amount of at least one enzyme for processing the expressed polypeptide to generate the activated truncated form of PDGF-D.

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This aspect also includes a method for selectively activating a polypeptide having a growth factor activity. This method comprises the step expressing an expression vector comprising a polynucleotide encoding a polypeptide having a growth factor

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activity, a CUB domain and a proteolytic site between the polypeptide and the CUB domain, and supplying a proteolytic amount of at least one enzyme for processing the expressed polypeptide to generate the activated polypeptide having a growth factor activity.

In addition, this aspect includes the isolation of a nucleic acid molecule which codes for a polypeptide having the biological activity of PDGF-D and a polypeptide thereof which comprises a proteolytic site having the amino acid sequence RKSR (SEQ ID NO:9) or a structurally conserved amino acid sequence thereof.

Also this aspect includes an isolated dimer comprising an activated monomer of PDGF-D and an activated monomer of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-D, PDGF-A, PDGF-B, PDGF-C or PlGF linked to a CUB domain, or alternatively, an activated monomer of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-D, PDGF-A, PDGF-B or PlGF and an activated monomer of PDGF-D linked to a CUB domain. The isolated dimer may or may not include a proteolytic site between the activator monomer and the CUB domain linkage.

Polynucleotides of the invention such as those described above, fragments of those polynucleotides, and variants of those polynucleotides with sufficient similarity to the non-coding strand of those polynucleotides to hybridize thereto under stringent conditions all are useful for identifying, purifying, and isolating polynucleotides encoding other, non-human, mammalian forms of PDGF-D. Thus, such polynucleotide fragments and variants are intended as aspects of the invention. Exemplary stringent hybridization conditions are as follows: hybridization at 42°C in 5X SSC, 20 mM NaPO₄, pH 6.8, 50% formamide; and washing at 42°C in 0.2X SSC. Those skilled in the art understand that it is desirable to vary these conditions empirically based on the length and the GC nucleotide base content of the sequences to be hybridized, and that formulas for determining such variation exist. See for example Sambrook et al, "Molecular Cloning: A

Laboratory Manual", Second Edition, pages 9.47-9.51, Cold Spring Harbor, New York: Cold Spring Harbor Laboratory (1989).

Moreover, purified and isolated polynucleotides encoding other, non-human, mammalian PDGF-D forms also are aspects of the invention, as are the polypeptides encoded thereby and antibodies that are specifically immunoreactive with the non-human PDGF-D variants. Thus, the invention includes a purified and isolated mammalian PDGF-D polypeptide and also a purified and isolated polynucleotide encoding such a polypeptide.

It will be clearly understood that nucleic acids and polypeptides of the invention may be prepared by synthetic means or by recombinant means, or may be purified from natural sources.

It will be clearly understood that for the purposes of this specification the word "comprising" means "included but not limited to". The corresponding meaning applies to the word "comprises".

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 (SEQ ID NO:1) shows a nucleotide sequence that includes a cDNA sequence encoding the C-terminal part of human PDGF-D (hPDGF-D). The nucleotides which encode for the partial fragment of hPDGF-D are 1 to 198. The deduced partial amino acid sequence of hPDGF-D (66 amino acid residues-SEQ ID NO:2) derived from nucleotides 1 to 198 of Figure 1 is shown in Figure 2;

Figure 3 (SEQ ID NO:3) shows an extended sequence of a partial human cDNA encoding for the hPDGF-D. The translated cDNA sequence is from nucleotide 1 to 600. The deduced partial amino acid sequence of hPDGF-D (200 residues-SEQ ID NO:4) derived from nucleotides 1 to 600 of Figure 3 is shown in Figure 4;

Figure 5 shows a still further extended nucleotide sequence of a partial human cDNA. The nucleotides which encode for the 5'-truncated full-length hPDGF-D are 1 to 966 (SEQ ID NO:5). The deduced partial amino acid sequence of hPDGF-D (322 residues-SEQ

5 ID NO:6) derived from nucleotides 1 to 966 of Figure 5 is shown in Figure 6;

10 Figure 7 (SEQ ID NO:7) shows the complete nucleotide sequence of cDNA encoding a hPDGF-D(1116 bp) and the deduced amino acid sequence of full-length hPDGF-D encoded thereby which consists of 371 amino acid residues (Figure 8-SEQ ID NO:8);

15 Figure 9 shows an amino acid sequence alignment of the PDGF/VEGF-homology domain in hPDGF-D with several growth factors belonging to the VEGF/PDGF family (SEQ ID NOs:10-18, respectively);

20 Figure 10 shows a phylogenetic tree of several growth factors belonging to the VEGF/PDGF family;

25 Figure 11 provides the amino acid sequence alignment of the CUB domain present in hPDGF-D (SEQ ID NO:19) and other CUB domains present in human bone morphogenic protein-1 (hBMP-1, 3 CUB domains CUB1-3) (SEQ ID NOs:20-22, respectively) and in human neuropilin-1 (2 CUB domains) (SEQ ID NOs:23-24, respectively);

30 Figure 12 shows that conditioned medium(CM)containing truncated PDGF-D stimulates tyrosine phosphorylation of PDGF beta-receptors in PAE-1 cells; and

35 Figure 13 provides a graphical representation of results which show that conditioned medium(CM)containing truncated PDGF-D competes for binding with PDGF-BB homodimers for the PDGF beta-receptors, but not with PDGF-AA homodimers for the PDGF alpha-receptors.

40 DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

45 Figure 1 shows a nucleotide sequence of human cDNA which encodes a C-terminal portion of a novel growth factor, referred to herein as PDGF-D (formerly VEGF-G). PDGF-D is a new member of the VEGF/PDGF family. The nucleotide sequence of Figure 1 (SEQ ID NO:1) was derived from a human EST sequence (id. AI488780) in the dbEST database at the NCBI in Washington, DC. The nucleotides

1 to 198 of the cDNA of Figure 1 (SEQ ID NO:1) encodes a 66 amino acid polypeptide (Figure 2-SEQ ID NO:2) which shows some sequence similarity to the known members of the VEGF/PDGF family.

The amino acid sequence of the polypeptide encoded by the nucleotides 1 to 198 of the polynucleotide of Figure 1 (SEQ ID NO:1) is shown in Figure 2 (SEQ ID NO:2).

To generate more sequence information on human PDGF-D, a human fetal lung λ gt10 cDNA library was screened using a 327 bp polymerase chain reaction (PCR)-generated probe, based on the originally identified EST sequence. The probe was generated from DNA from a commercially available human fetal lung cDNA library (Clontech) which was amplified by PCR using two primers derived from the identified EST (A1488780). The primers were:

5'-GTCGTGGAAGTGTCACTGG (forward) (SEQ ID NO:26) and

5'-CTCAGCAACCACTTGTGTTC (reverse) (SEQ ID NO:27).

The amplified 327 bp fragment was cloned into the pCR2.1 vector (Invitrogen). Nucleotide sequencing verified that the insert corresponded to the EST. The screen identified several positive clones. The inserts from two of these clones, clones 5 and 8 were subcloned into pBluescript and subjected to nucleotide sequencing using internal or vector-specific primers. The nucleotide sequences determined were identical in both clones and are shown in Figure 3 (SEQ ID NO:3). The coding region of the 690 bp polynucleotide is nucleotides 1-600 (SEQ ID NO:3) that encodes for a large portion of hPDGF-D with the exception of the 5'-end. This portion of hPDGF-D includes the bioactive fragment of hPDGF-D. The deduced partial amino acid sequence of hPDGF-D (200 residues-SEQ ID NO:4) derived from nucleotides 1 to 600 of Figure 3 (SEQ ID NO:3) is shown in Figure 4 (SEQ ID NO:4).

Extended nucleotide sequencing of the isolated human PDGF-D cDNA clones from this human fetal lung cDNA library has provided additional sequence. Figure 5 (SEQ ID NO:5) shows a nucleotide sequence of a partial human cDNA (1934 bp) that encodes hPDGF-D.

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The coding region of the 1934 bp polynucleotide is nucleotides 1 to 966 that encodes for hPDGF-D except for the most 5'-end of the polypeptide. The deduced partial amino acid sequence of hPDGF-D (322 residues-SEQ ID NO:6) derived from nucleotides 1 to 966 of Figure 5 (SEQ ID NO:5) is shown in Figure 6 (SEQ ID NO:6).

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Figure 7 (SEQ ID NO:7) shows a polynucleotide sequence of cDNA encoding a full-length hPDGF-D. The region encoding PDGF-D is 1116 bp. The deduced amino acid sequence of full-length hPDGF-D is 371 amino acid residues (Figure 8-SEQ ID NO:8).

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10 The sequence for the 5' end of full-length PDGF-D was obtained using Rapid Amplification of cDNA Ends (RACE) PCR, and clones containing cDNA from the human heart (Marathon-Ready™ cDNA, Clontech, Cat# 7404-1). These cDNA clones have an adaptor sequence attached to the 5' end of each clone, including a site for primer called Adaptor Primer 1 (Clontech: 5'9 CCATCCTAATACGACTCACTATAGGGC 3'9) (SEQ ID NO:28). This primer and a second primer 5'AGTGGGATCCGTTACTGA TGGAGAGTTAT 3' (SEQ ID NO:29) were used to amplify the sequence found at the 5' end of PDGF-D. In the PCR reaction a special polymerase mix was used (Advantage«-GC cDNA PCR Kit, Clontech, Cat# K1907-1). The reaction mix included (in microliters):

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Adaptor Primer 1	1
Gene specific primer	1
Template (Human Heart cDNA)	5
GC-Melt (from the K1907-1 Kit)	5
5xGC cDNA PCR Reaction Buffer	10
50x dNTP mix	1
Sterile H2O	27
Total	50

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The 5' end of PDGF-D was amplified for 31 cycles, five cycles consisted of 45 seconds denaturation at 94°C and four minutes extension at 72°C, five cycles consisted of 45 seconds

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denaturation at 94°C and four minutes extension at 70°C, and five cycles consisted of 45 seconds denaturation at 94°C and four minutes extension at 68°C and an initial denaturation step at 94°C for two minutes. From this PCR, an approximately 790 bp long product was obtained. This product was run on a 1% agarose gel, purified (QIAquick gel extraction Kit, Qiagen, Cat # 28706) from the gel, cloned into a vector (TOPO TA Cloning Kit, Invitrogen) and transformed into bacteria (E.Coli). Transformed bacteria were plated, and incubated at 37°C overnight. Single colonies were picked and grown in fresh media overnight. Plasmids were prepared (QIAprep Spin Miniprep Kit, Qiagen, Cat# 27106) and sequenced with the plasmid primers, T7 and M13R. The result of this sequencing was that 312 bp of previously unknown PDGF-D sequence was obtained. The rest of the sequence (478 bp) was identical with previously obtained sequence from other PDGF-D cDNA clones.

Figure 9 shows the amino acid sequence alignment of the PDGF/VEGF-homology domain of PDGF-D (found in the C-terminal region of the polypeptide) with the PDGF/VEGF-homology domains of PDGF/VEGF family members, VEGF₁₆₅, PlGF-2, VEGF-B₁₆₇, Pox Orf VEGF, VEGF-C, VEGF-D, PDGF-A and PDGF-B (SEQ ID NOS:10-18, respectively). Some of the amino acid sequences in the N- and C-terminal regions in VEGF-C and VEGF-D have been deleted in this figure. Gaps were introduced to optimize the alignment. This alignment was generated using the MEGALIGN alignment tool based on the method of J. Hein, (Methods Enzymol. 1990 183 626-45) The PAM 250 residue weight table is used with a gap penalty of eleven and a gap length penalty of three and a K-tuple value of two in the pairwise alignments. The alignment is then refined manually, and the number of identities are estimated in the regions available for a comparison. The boxed residues indicate amino acids which match the VEGF-D within two distance units.

The alignment shows that PDGF-D has the expected pattern of invariant cysteine residues, a hallmark of members of this family,

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with two exceptions. The first exception occurs between cysteine 3 and 4. Normally these two cysteines are spaced by 2 residues and with PDGF-D there is an insertion of three extra amino acids (NCA). This feature of the sequence in PDGF-D was highly unexpected. The second is that the invariant fifth cysteine found in the other members of the PDGF/VEGF family is not conserved in PDGF-D. This feature is unique to PDGF-D.

Based on the amino acid sequence alignments in Figure 9, a phylogenetic tree was constructed and is shown in Figure 10. The data show that the PVHD of PDGF-D is closely related to the PVHDs of VEGF-C and VEGF-D.

CUB Domain

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The N-terminal region of the partial PDGF-D amino acid sequence of Figure 11 (residues 54-171) (SEQ ID NO:8) has second distinct protein domain which is referred to as a CUB domain (Bork and Beckmann, J. Mol. Biol., 1993 231, 539-545). This domain of about 115 amino acids was originally identified in complement factors C1r/C1s, but has recently been identified in several other extracellular proteins including signaling molecules such as bone morphogenic protein 1 (BMP-1) (Wozney *et al.*, Science, 1988 242, 1528-1534) as well as in several receptor molecules such as neuropilin-1 (NP-1) (Soker *et al.*, Cell, 1998 92 735-745). The functional roles of CUB domains are not clear but they may participate in protein-protein interactions or in interactions with carbohydrates including heparin sulfate proteoglycans. These interactions may play a role the proteolytic activation of PDGF-D.

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As shown in Figure 11, the amino acid sequences from several CUB-containing proteins were aligned. The results show that the single CUB domain in human PDGF-D (SEQ ID NO:19) displays a significant identify with the most closely related CUB domains. Sequences from human BMP-1, with 3 CUB domains (CUBs1-3) (SEQ ID NOs:20-22, respectively) and human neuropilin-1 with 2 CUB domains

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(CUBs1-2) (SEQ ID NOs:23-24, respectively) are shown. This alignment was generated as described above.

Example 1: Expression of PDGF-D transcripts

To investigate the tissue expression of PDGF-D in several human tissues, a Northern blot was done using a commercial Multiple Tissue Northern blot (MTN, Clontech). The blots were hybridized at according to the instructions from the supplier using ExpressHyb solution at 68°C for one hour (high stringency conditions), and probed with the 327 bp PCR-generated probe from the human fetal lung cDNA library (see description above). The blots were subsequently washed at 50°C in 2X SSC with 0.05% SDS for 30 minutes and at 50°C in 0.1X SSC with 0.1% SDS for an additional 40 minutes. The blots were then put on film and exposed at -70°C. The results, summarized in Table 1, showed that expression of PDGF-D transcripts were most abundant in heart, pancreas and ovary while lower expression levels were seen in placenta, liver, kidney, prostate and testis. The human PDGF-D transcript was around 4 kb in length.

Table 1. Relative expression levels of PDGF-D transcripts in several human tissues as determined by Northern blot analysis

<u>Tissue level*</u>	<u>Expression</u>
Heart	+++++
Brain	n.d.
Placenta	++
Lung	+
Liver	++
Skeletal muscle	n.d.
Kidney	++
Pancreas	++++
Spleen	+
Thymus	+
Prostate	++
Testis	+++
Ovary	+++++

Small intestine ++
Colon +
Peripheral blood +
leucocytes

The relative intensities of the bands were visually determined (+++++) highest expression and (+) lowest expression; n.d. not detected

Example 2: Receptor binding properties of a truncated PDGF-D

To assess the interactions between a truncated PDGF-D and the VEGF receptors, truncated PDGF-D was tested for its capacity to bind to soluble Ig-fusion proteins containing the extracellular domains of human VEGFR-1, VEGFR-2 and VEGFR-3 (Olofsson et al., Proc. Natl. Acad. Sci. USA, 1998 95 11709-11714). An expression vector encoding the PDGF/VEGF homology domain of PDGF-D was generated in the vector pSecTag (Invitrogen). The primers 5'-CCCAAGCTTGAAGATCTTGAGAATAT 3' (forward) (SEQ ID NO:30) and 5'-TGCTCTAGATCGAGGTGGTCTT 3' (reverse) (SEQ ID NO:31) were used to amplify a 429 bp fragment (nucleotides 556 to 966 in Figure 5) (SEQ ID NO:5) encoding amino acid residues 186 to 322 of Figure 6 (SEQ ID NO:6). The fragment was subsequently cloned into a HindIII and XbaI digested expression vector. COS cells were transfected with the expression vector encoding truncated PDGF-D or a control vector using calcium phosphate precipitation. The expressed polypeptide included a C-terminal c-myc tag and a 6X His tag (both derived from the pSecTag vector).

The Ig-fusion proteins, designated VEGFR-1-Ig, VEGFR-2-Ig and VEGFR-3-Ig, were transiently expressed in human 293 EBNA cells. All Ig-fusion proteins were human VEGFRs. Cells were incubated for 24 hours after transfection, washed with Dulbecco's Modified Eagle Medium (DMEM) containing 0.2% bovine serum albumin (BSA) and starved for 24 hours. The fusion proteins were then precipitated from the clarified conditioned medium using protein A-Sepharose beads (Pharmacia). The beads were combined with 100 microliters

5 of 10X binding buffer (5% BSA, 0.2% Tween 20 and 10 µg/ml heparin)
and 900 microliter of conditioned medium prepared from the COS
10 cells transfected with the expression vector for truncated PDGF-D
or the control vector. The cells were then metabolically labeled
5 with ³⁵S-cysteine and methionine (Promix, Amersham) for 4 to 6
hours. After 2.5 hours, at room temperature, the Sepharose beads
15 were washed 3 times with binding buffer at 4°C, once with
phosphate buffered saline (PBS) and boiled in SDS-PAGE buffer.
Labeled proteins that were bound to the Ig-fusion proteins were
10 analyzed by SDS-PAGE under reducing conditions. Radiolabeled
proteins were detected using a phosphorimager analyzer and/or on
20 film. In all these analyses, radiolabeled PDGF-D failed to show
any interaction with any of the VEGF receptors. These results
indicate that secreted truncated PDGF-D does not bind to VEGF
25 15 receptors R1, R2 and R3.

Example 3: PDGF beta-receptor Phosphorylation

30 To test if PDGF-D causes increased phosphorylation of the
PDGF beta-receptor, truncated PDGF-D was tested for its capacity
20 to bind to the PDGF beta-receptor and stimulate increased
phosphorylation. Serum-starved porcine aortic endothelial-1 (PAE-
1) cells stably expressing the human PDGF beta-receptor (Eriksson
35 et al., EMBO J, 1992, 11, 543-550) were incubated on ice for 90
minutes with a solution of conditioned media mixed with an equal
25 volume of PBS supplemented with 1 mg/ml BSA. The conditioned
media was prepared from COS cells transfected with expression
40 vectors for PDGF-A or truncated PDGF-D (as constructed in Example
1), or a mock control vector. Twenty-four hours after
transfection, the medium was replaced by serum-free medium
30 containing 1mg/ml serum albumin. Conditioned medium was harvested
after an additional 48 hours of incubation. Sixty minutes after
the addition of the conditioned media, the cells were lysed in
45 lysis buffer (20 mM tris-HCl, pH 7.5, 0.5% Triton X-100, 0.5%

5 deoxycholic acid, 10 mM EDTA, 1 mM orthovanadate, 1 mM PMSF 1%
Trasylol). The PDGF beta-receptors were immunoprecipitated from
10 cleared lysates with rabbit antisera against the human PDGF beta-
receptor (Eriksson et al., EMBO J, 1992 11 543-550). The
5 precipitated receptors were applied to a SDS-PAGE gel. After SDS
gel electrophoresis, the precipitated receptors were transferred
15 to nitrocellulose filters, and the filters were probed with anti-
phosphotyrosine antibody PY-20, (Transduction Laboratories). The
filters were then incubated with horseradish peroxidase-conjugated
20 anti-mouse antibodies. Bound antibodies were detected using
enhanced chemiluminescence (ECL, Amersham Inc). The filters were
then stripped and reprobed with the PDGF beta-receptor rabbit
antisera, and the amount of receptors was determined by incubation
with horseradish peroxidase-conjugated anti-rabbit antibodies.
25 Bound antibodies were detected using enhanced chemiluminescence
(ECL, Amersham Inc). The probing of the filters with PDGF beta
receptor antibodies confirmed that equal amounts of the receptor
were present in all lanes. Human recombinant PDGF-BB (100ng/ml)
30 and untreated cells were included in the experiment as a control.
20 Figure 11 shows that truncated PDGF-D containing conditioned
medium stimulated PDGF beta-receptor tyrosine phosphorylation.
This indicates that truncated PDGF-D is a PDGF beta-receptor
35 ligand/agonist.

25 Example 4: Competitive Binding Assay

40 Next, truncated PDGF-D was tested for its capacity to bind
to the human PDGF beta-receptor by analyzing its ability to
compete with PDGF-BB for binding to the PDGF beta-receptor. The
binding experiments were performed on porcine aortic endothelial-1
45 (PAE-1) cells stably expressing the human PDGF alpha- and beta-
receptors, respectively (Eriksson et al., EMBO J, 1992, 11, 543-
550). Binding experiments were performed essentially as in Heldin
et al. (EMBO J, 1988, 7 1387-1393). Conditioned media from COS

5 cells expressing PDGF-A, truncated PDGF-D, or mock control,
respectively, was diluted with an equal volume of BSA/PBS and
10 mixed with 100 ng/ml of ¹²⁵I-PDGF-BB (beta-receptor ligand) or of
¹²⁵I-PDGF-AA (alpha-receptor ligand) in binding buffer (PBS
5 containing 1 mg/ml of BSA). Two separate sets of conditioned
media from these COS cells were analyzed. Aliquots were incubated
15 with the receptor expressing PAE-1 cells plated in 24-well culture
dishes on ice for 90 minutes. After three washes with binding
buffer, cell-bound ¹²⁵I-PDGF-BB or ¹²⁵I-PDGF-AA was extracted by
10 lysis of cells in 20 mM Tris-HCl, pH 7.5, 10% glycerol, 1% Triton
X-100. The amount of cell bound radioactivity was determined in
20 a gamma-counter. Figure 12 provides a graphical representation
of results which show that conditioned medium containing truncated
PDGF-D competes for binding with PDGF-BB homodimers for the PDGF
25 15 beta-receptors, but not with PDGF-AA homodimers for the PDGF
alpha-receptors.

30 PDGF-D does not bind to any of the known VEGF receptors.
PDGF-D is the only VEGF family member, thus far, which can bind
20 to and increase phosphorylation of the PDGF beta-receptor. These
characteristics indicate that the truncated form of PDGF-D may not
be a VEGF family member, but instead a novel PDGF. Furthermore,
35 the full length protein is likely to be a latent growth factor
that needs to be activated by proteolytic processing to release
25 the active PDGF/VEGF homology domain. The N-terminal CUB domain
may be expressed as an inhibitory domain which might be used to
40 localize this latent growth factor in some extracellular
compartment (for example the extracellular matrix) and which is
removed by limited proteolysis when need, for example during
45 30 embryonic development, tissue regeneration, tissue remodelling
including bone remodelling, active angiogenesis, tumor
progression, tumor invasion, metastasis formation and/or wound
healing.

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BIOASSAYS TO DETERMINE THE FUNCTION OF PDGF-D

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Assays are conducted to evaluate whether PDGF-D has similar activities to PDGF-A, PDGF-B, VEGF, VEGF-B, VEGF-C and/or VEGF-D in relation to growth and/or motility of connective tissue cells, fibroblasts, myofibroblasts and glial cells; to endothelial cell function; to angiogenesis; and to wound healing. Further assays may also be performed, depending on the results of receptor binding distribution studies.

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I. Mitogenicity of PDGF-D for Endothelial Cells

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To test the mitogenic capacity of PDGF-D for endothelial cells, the PDGF-D polypeptide is introduced into cell culture medium containing 5% serum and applied to bovine aortic endothelial cells (BAEs) propagated in medium containing 10% serum. The BAEs are previously seeded in 24-well dishes at a density of 10,000 cells per well the day before addition of the PDGF-D. Three days after addition of this polypeptide the cells were dissociated with trypsin and counted. Purified VEGF is included in the experiment as positive control.

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II. Mitogenicity of PDGF-D for Fibroblasts

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To test the mitogenic capacity of PDGF-D for fibroblasts, different concentrations of truncated homodimers of PDGF-DD or PDGF-AA (as control) are added to serum starved human foreskin fibroblasts in the presence of 0.2 μ M [3H]thymidine. The fibroblasts are then incubated for 24 hours with 1 ml of serum-free medium supplemented with 1 mg/ml BSA. After trichloroacetic acid (TCA) precipitation, the incorporation of [3H]thymidine into DNA is determined using a beta-counter. The assay is performed essentially as described in Mori et al., J. Biol. Chem., 1991 266 21158-21164.

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III. Assays of Endothelial Cell Functiona) *Endothelial cell proliferation*

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Endothelial cell growth assays are performed by methods well known in the art, e.g. those of Ferrara & Henzel, Nature, 1989 380 439-443, Gospodarowicz et al., Proc. Natl. Acad. Sci. USA, 1989 86 7311-7315, and/or Claffey et al., Biochem. Biophys. Acta, 1995 1246 1-9.

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b) *Cell adhesion assay*

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The effect of PDGF-D on adhesion of polymorphonuclear granulocytes to endothelial cells is tested.

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c) *Chemotaxis*

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The standard Boyden chamber chemotaxis assay is used to test the effect of PDGF-D on chemotaxis.

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d) *Plasminogen activator assay*

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Endothelial cells are tested for the effect of PDGF-D on plasminogen activator and plasminogen activator inhibitor production, using the method of Pepper et al., Biochem. Biophys. Res. Commun., 1991 181 902-906.

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e) *Endothelial cell Migration assay*

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The ability of PDGF-D to stimulate endothelial cells to migrate and form tubes is assayed as described in Montesano et al., Proc. Natl. Acad. Sci. USA, 1986 83 7297-7301. Alternatively, the three-dimensional collagen gel assay described in Joukov et al., EMBO J., 1996 15 290-298 or a gelatinized membrane in a modified Boyden chamber (Glaser et al., Nature, 1980 288 483-484) may be used.

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IV. Angiogenesis Assay

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5 The ability of PDGF-D to induce an angiogenic response in
chick chorioallantoic membrane is tested as described in Leung et
10 al., Science, 1989 246 1306-1309. Alternatively the rat cornea
assay of Rastinejad et al., Cell, 1989 56 345-355 may be used;
5 this is an accepted method for assay of *in vivo* angiogenesis, and
the results are readily transferrable to other *in vivo* systems.

15 V. Wound Healing

The ability of PDGF-D to stimulate wound healing is tested
10 in the most clinically relevant model available, as described in
Schilling et al., Surgery, 1959 46 702-710 and utilized by Hunt
20 et al., Surgery, 1967 114 302-307.

25 VI. The Haemopoietic System

15 A variety of *in vitro* and *in vivo* assays using specific cell
populations of the haemopoietic system are known in the art, and
are outlined below. In particular a variety of *in vitro* murine
stem cell assays using fluorescence-activated cell sorter to
30 purified cells are particularly convenient:

20 a) Repopulating Stem Cells

35 These are cells capable of repopulating the bone marrow of
lethally irradiated mice, and have the Lin⁻, Rh^{h1}, Ly-6A/E⁺, c-kit⁺
phenotype. PDGF-D is tested on these cells either alone, or by
25 co-incubation with other factors, followed by measurement of
40 cellular proliferation by ³H-thymidine incorporation.

b) Late Stage Stem Cells

45 These are cells that have comparatively little bone marrow
30 repopulating ability, but can generate D13 CFU-S. These cells
have the Lin⁻, Rh^{h1}, Ly-6A/E⁺, c-kit⁺ phenotype. PDGF-D is
incubated with these cells for a period of time, injected into

lethally irradiated recipients, and the number of D13 spleen colonies enumerated.

c) *Progenitor-Enriched Cells*

These are cells that respond *in vitro* to single growth factors and have the Lin⁻, Rh^{h1}, Ly-6A/E⁺, c-kit⁺ phenotype. This assay will show if PDGF-D can act directly on haemopoietic progenitor cells. PDGF-D is incubated with these cells in agar cultures, and the number of colonies present after 7-14 days is counted.

VII. Atherosclerosis

Smooth muscle cells play a crucial role in the development or initiation of atherosclerosis, requiring a change of their phenotype from a contractile to a synthetic state. Macrophages, endothelial cells, T lymphocytes and platelets all play a role in the development of atherosclerotic plaques by influencing the growth and phenotypic modulations of smooth muscle cell. An *in vitro* assay using a modified Rose chamber in which different cell types are seeded on to opposite cover slips measures the proliferative rate and phenotypic modulations of smooth muscle cells in a multicellular environment, and is used to assess the effect of PDGF-D on smooth muscle cells.

VIII. Metastasis

The ability of PDGF-D to inhibit metastasis is assayed using the Lewis lung carcinoma model, for example using the method of Cao et al., J. Exp. Med., 1995 182 2069-2077.

IX. Migration of Smooth Muscle Cells

The effects of the PDGF-D on the migration of smooth muscle cells and other cells types can be assayed using the method of Koyama et al., J. Biol. Chem., 1992 267 22806-22812.

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X. Chemotaxis

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The effects of the PDGF-D on chemotaxis of fibroblast, monocytes, granulocytes and other cells can be assayed using the method of Siegbahn *et al.*, J. Clin. Invest., 1990 85 916-920.

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XI. PDGF-D in Other Cell Types

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The effects of PDGF-D on proliferation, differentiation and function of other cell types, such as liver cells, cardiac muscle and other cells, endocrine cells and osteoblasts can readily be assayed by methods known in the art, such as ³H-thymidine uptake by *in vitro* cultures.

XII. Construction of PDGF-D Variants and Analogues

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PDGF-D is a member of the PDGF family of growth factors which exhibits a high degree of homology to the other members of the PDGF family. PDGF-D contains seven conserved cysteine residues which are characteristic of this family of growth factors. These conserved cysteine residues form intra-chain disulfide bonds which produce the cysteine knot structure, and inter-chain disulfide bonds that form the protein dimers which are characteristic of members of the PDGF family of growth factors. PDGF-D interacts with a protein tyrosine kinase growth factor receptor.

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In contrast to proteins where little or nothing is known about the protein structure and active sites needed for receptor binding and consequent activity, the design of active mutants of PDGF-D is greatly facilitated by the fact that a great deal is known about the active sites and important amino acids of the members of the PDGF family of growth factors.

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Published articles elucidating the structure/activity relationships of members of the PDGF family of growth factors include for PDGF: Oestman *et al.*, J. Biol. Chem., 1991 266 10073-10077; Andersson *et al.*, J. Biol. Chem., 1992 267 11260-1266; Oefner *et al.*, EMBO J., 1992 11 3921-3926; Flemming *et al.*,

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Molecular and Cell Biol., 1993 13 4066-4076 and Andersson et al., Growth Factors, 1995 12 159-164; and for VEGF: Kim et al., Growth Factors, 1992 7 53-64; Pötgens et al., J. Biol. Chem., 1994 269 32879-32885 and Claffey et al., Biochem. Biophys. Acta, 1995 1246 1-9. From these publications it is apparent that because of the eight conserved cysteine residues, the members of the PDGF family of growth factors exhibit a characteristic knotted folding structure and dimerization, which result in formation of three exposed loop regions at each end of the dimerized molecule, at which the active receptor binding sites can be expected to be located.

Based on this information, a person skilled in the biotechnology arts can design PDGF-D mutants with a very high probability of retaining PDGF-D activity by conserving the eight cysteine residues responsible for the knotted folding arrangement and for dimerization, and also by conserving, or making only conservative amino acid substitutions in the likely receptor sequences in the loop 1, loop 2 and loop 3 region of the protein structure.

The formation of desired mutations at specifically targeted sites in a protein structure is considered to be a standard technique in the arsenal of the protein chemist (Kunkel et al., Methods in Enzymol., 1987 154 367-382). Examples of such site-directed mutagenesis with VEGF can be found in Pötgens et al., J. Biol. Chem., 1994 269 32879-32885 and Claffey et al., Biochem. Biophys. Acta, 1995 1246 1-9. Indeed, site-directed mutagenesis is so common that kits are commercially available to facilitate such procedures (e.g. Promega 1994-1995 Catalog., Pages 142-145).

The connective tissue cell, fibroblast, myofibroblast and glial cell growth and/or motility activity, the endothelial cell proliferation activity, the angiogenesis activity and/or the wound healing activity of PDGF-D mutants can be readily confirmed by well established screening procedures. For example, a procedure

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analogous to the endothelial cell mitotic assay described by Claffey et al., (Biochem. Biophys. Acta., 1995 1246 1-9) can be used. Similarly the effects of PDGF-D on proliferation of other cell types, on cellular differentiation and on human metastasis can be tested using methods which are well known in the art.

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The foregoing description and examples have been set forth merely to illustrate the invention and are not intended to be limiting. Since modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art, the invention should be construed broadly to include all variations falling within the scope of the appended claims and equivalents thereof.

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Claims

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What is claimed is:

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1. An isolated nucleic acid molecule comprising a polynucleotide sequence having at least 85% identity with at least nucleotides 1 to 600 of the sequence set out in Figure 3 (SEQ ID NO:3), at least nucleotides 1 to 966 of the sequence set out in Figure 5 (SEQ ID NO:5), at least nucleotides 176-1288 of Figure 7 (SEQ ID NO:5) or at least nucleotides 938 to 1288 set out in Figure 7 (SEQ ID NO:7).

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2. An isolated nucleic acid molecule according to claim 1, wherein the sequence identity is at least 90%.

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3. An isolated nucleic acid molecule according to claim 1, wherein the sequence identity is at least 95%.

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4. An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid is a cDNA.

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5. An isolated nucleic acid molecule according to claim 1, wherein said nucleic acid is a mammalian polynucleotide.

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7. An isolated nucleic acid molecule which encodes a polypeptide molecule comprising an amino acid sequence having at least 85% identity with the amino acid sequence of Figure 4 (SEQ ID NO:4), the amino acid sequence of Figure 6 (SEQ ID NO:6), or the amino acid sequence of Figure 8 (SEQ ID NO:8), or a fragment or analog thereof having the biological activity of PDGF-D.

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8. An isolated nucleic acid molecule according to claim 7, wherein the amino acid sequence identity is at least 90%.

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9. An isolated nucleic acid molecule according to claim 7, wherein the amino acid sequence identity is at least 95%.

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10. An isolated nucleic acid molecule which codes for a polypeptide which comprises the amino acid sequence

PXCLLVXRCGGNCXC (SEQ ID NO:25).

20

11. An isolated polynucleotide, comprising a polynucleotide sequence having at least 85% identity with at least nucleotides 1 to 600 of the sequence set out in Figure 3 (SEQ ID NO:3), at least nucleotides 1 to 966 of the sequence set out in Figure 5 (SEQ ID NO:5), at least nucleotides 176-1288 of Figure 7 (SEQ ID NO:5) or at least nucleotides 938 to 1288 set out in Figure 7 (SEQ ID NO:7), or a polynucleotide which hybridizes under stringent conditions with at least one of said DNA sequences.

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12. A vector comprising a nucleic acid according to claim 1 or claim 11, which nucleic acid is operably linked with a promoter sequence.

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13. A vector according to claim 12, wherein said vector is a eukaryotic vector.

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14. A vector according to claim 12, wherein said vector is a prokaryotic vector.

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15. A vector according to claim 12, wherein said vector is a plasmid.

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16. A vector according to claim 12, wherein said vector is a baculovirus vector.

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17. A method of making a vector which expresses a polypeptide comprising an amino acid sequence having at least 85% identity with the amino acid sequence of Figure 4 (SEQ ID NO:4), the amino acid sequence of Figure 6 (SEQ ID NO:6), or the amino acid sequence of Figure 8 (SEQ ID NO:8), or fragment or analog thereof having the biological activity of PDGF-D, said method comprising incorporating an isolated nucleic acid according to claim 1, claim 7, claim 10 or claim 11, into said vector in operatively linked relation with a promoter.

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18. A host cell transformed or transfected with a vector according to claim 12.

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19. A host cell according to claim 18, wherein said host cell is a eukaryotic cell.

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20. A host cell according to claim 18, wherein said host cell is a COS cell.

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21. A host cell according to claim 18, wherein said host cell is a prokaryotic cell.

22. A host cell according to claim 18, wherein said host cell is a 293EBNA cell.

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23. A host cell according to claim 18, wherein said host cell is an insect cell.

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24. A host cell transformed or transfected with a vector comprising a nucleic acid sequence according to claim 1 or claim

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11, operatively linked to a promoter, such that said host cell expresses a polypeptide comprising an amino acid sequence having at least 85% identity with the amino acid sequence of Figure 4 (SEQ ID NO:4), the amino acid sequence of Figure 6 (SEQ ID NO:6), or the amino acid sequence of Figure 8 (SEQ ID NO:8), or a fragment or analog thereof having the biological activity of PDGF-D.

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25. An isolated polypeptide comprising at least 85% identity with the amino acid sequence of Figure 4 (SEQ ID NO:4), the amino acid sequence of Figure 6 (SEQ ID NO:6), or the amino acid sequence of Figure 8 (SEQ ID NO:8), or a fragment or analog thereof having the biological activity of PDGF-D.

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26. An isolated polypeptide according to claim 25, wherein said polypeptide is a human polypeptide.

30

27. An isolated polypeptide according to claim 25, wherein said polypeptide has the ability to stimulate and/or enhance proliferation and/or differentiation and/or growth and/or motility of cells expressing a PDGF-D receptor.

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28. An isolated polypeptide according to claim 27, wherein the cells are selected from the group consisting of endothelial cells, connective tissue cells, myofibroblasts and glial cells.

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29. An isolated polypeptide produced by expression of a polynucleotide comprising the polynucleotide sequence having at least 85% identity with at least nucleotides 1 to 600 of the sequence set out in Figure 3 (SEQ ID NO:3), at least nucleotides 1 to 966 of the sequence set out in Figure 5 (SEQ ID NO:5), at least nucleotides 176-1288 of Figure 7 (SEQ ID NO:5) or at least nucleotides 938 to 1288 set out in Figure 7 (SEQ ID NO:7), or a

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polynucleotide which hybridizes under stringent conditions with at least one of said DNA sequences.

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30. An isolated polypeptide which comprises the characteristic sequence

PXCLLVXRCGGNCXC (SEQ ID NO:25).

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31. An isolated polypeptide dimer comprising a polypeptide according to claim 25 or claim 29.

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32. An isolated polypeptide dimer according to claim 31, wherein said polypeptide dimer is a homodimer of said polypeptide.

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33. An isolated polypeptide dimer according to claim 31, wherein said polypeptide dimer is a heterodimer of said polypeptide and VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-A, PDGF-B or PlGF.

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34. An isolated polypeptide dimer according to claim 31, wherein said polypeptide dimer is a disulfide-linked dimer.

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35. A pharmaceutical composition comprising an effective cell proliferation promoting amount of a polypeptide according to claim 25, claim 29 or claim 30, and at least one further growth factor selected from the group consisting of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-A, PDGF-B or PlGF.

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36. A pharmaceutical composition according to claim 35, further comprising heparin.

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37. A pharmaceutical composition comprising an effective cell proliferation promoting amount of an isolated polypeptide

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according to claim 25, claim 29 or claim 30, and at least one pharmaceutical carrier or diluent.

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38. A pharmaceutical composition according to claim 37, further comprising heparin.

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39. A pharmaceutical composition comprising an effective amount of an isolated polypeptide according to claim 25, claim 29 or claim 30 and heparin.

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40. A pharmaceutical composition comprising a PDGF receptor stimulating amount of an isolated polypeptide according to claim 25, claim 29 or claim 30, and at least one pharmaceutical carrier or diluent.

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41. A means for amplifying a polynucleotide according to claim 1 or claim 11 in a test sample, said means comprising at least one pair of primers complementary to a nucleic acid according to claim 1 or claim 11.

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42. A means for amplifying a polynucleotide as according to claim 1 or claim 11 in a test sample, said means comprising a polymerase and at least one pair of primers complementary to a nucleic acid according to claim 1 or claim 11, for amplifying the polynucleotide by polymerase chain reaction in order to facilitate a sequence comparison of the polynucleotide with the nucleic acid according to claim 1 or claim 11.

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43. An antibody specifically reactive with a polypeptide according to claim 25, claim 29 or claim 30.

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44. An antibody according to claim 43, wherein said antibody is a polyclonal antibody.

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45. An antibody according to claim 43, wherein said antibody is a monoclonal antibody.

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46. An antibody according to claim 45, wherein said antibody is a humanized antibody.

15

47. An antibody according to claim 44 or 45 or 46, wherein said antibody is labeled with a detectable label.

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48. An antibody according to claim 47, wherein said detectable label is radioactive isotope.

25

49. An antibody according to claim 45 or 46, wherein the antibody is modified by addition of cytotoxic or cytostatic drug.

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50. A method of making a polypeptide according to claim 25, claim 29 or claim 30, said method comprising the steps of:

culturing a host cell transformed or transfected with a vector comprising a polynucleotide encoding said polypeptide operably associated with a promoter sequence such that the nucleic acid sequence encoding said polypeptide is expressed; and

35

isolating said polypeptide from said host cell or from a growth medium in which said host cell is cultured.

40

51. A method of stimulating growth of connective tissue or wound healing in a mammal comprising the step of administering to said mammal an effective connective tissue or wound healing stimulating amount of the polypeptide according to claim 25, claim 29 or 30.

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52. A method for producing an activated truncated form of PDGF-D, comprising the steps of expressing an expression vector comprising a polynucleotide encoding a polypeptide as claimed in

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claim 25, claim 29 or claim 30 and supplying a proteolytic amount
of at least one enzyme for processing the expressed polypeptide
10 to generate the activated truncated form of PDGF-D.

15 53. A method for regulating receptor-binding specificity of
PDGF-D, comprising the steps of expressing an expression vector
comprising a polynucleotide encoding a polypeptide as claimed in
claim 25, claim 29 or claim 30 and supplying a proteolytic amount
of at least one enzyme for processing the expressed polypeptide
to generate the activated truncated form of PDGF-D.

20 54. A method for selectively activating a polypeptide having
a growth factor activity comprising the step expressing an
expression vector comprising a polynucleotide encoding a
25 polypeptide having a growth factor activity, a CUB domain and a
proteolytic site between the polypeptide and the CUB domain, and
supplying a proteolytic amount of at least one enzyme for
processing the expressed polypeptide to generate the activated
30 polypeptide having a growth factor activity.

35 55. An isolated polypeptide according to claim 25, claim 29
or claim 30 which comprises a proteolytic site having the amino
acid sequence RKSK or a structurally conserved amino acid sequence
thereof.

40 56. An isolated nucleic acid molecule according to claim 7
which codes for a polypeptide which comprises a proteolytic site
having the amino acid sequence RKSK or a structurally conserved
amino acid sequence thereof.

45 57. An isolated heterodimer comprising an activated monomer
of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-D, PDGF-A, PDGF-B or PlGF
and an activated monomer of PDGF-D linked to a CUB domain.

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58. An isolated heterodimer comprising an activated monomer of PDGF-D and an activated monomer of VEGF, VEGF-B, VEGF-C, VEGF-D, PDGF-D, PDGF-A, PDGF-B or PlGF linked to a CUB domain.

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59. An isolated heterodimer according to claim 57, further comprising a proteolytic site between the activator monomer and the CUB domain linkage.

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60. An isolated heterodimer according to claim 58, further comprising a proteolytic site between the activator monomer and the CUB domain linkage.

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61. A method of inducing PDGF beta-receptor activation, comprising the step of adding a PDGF beta-receptor stimulating amount of the polypeptide according to claim 25, claim 29 or claim 30.

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62. A method of inhibiting tumor growth of a tumor expressing PDGF-D in a mammal, comprising the step of administering to said mammal a PDGF-D inhibiting amount of a PDGF-D antagonist.

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63. A method of identifying specific types of human tumors, comprising the step of taking a sample of the tumor and testing for the expression of PDGF-D.

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64. A method for identifying an PDGF-D antagonist comprising:

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admixing a substantially purified preparation of an activated truncated form of PDGF-D with a test agent; and monitoring, by any suitable means, an inhibition in the biological activity of PDGF-D.

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65. A method for identifying an PDGF-D antagonist comprising:

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admixing a substantially purified preparation of an full-length PDGF-D with a test agent; and

monitoring, by any suitable means, an inhibition in the cleavage of the CUB domain from PDGF-D.

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66. A method of making a vector which expresses a polypeptide comprising an amino acid sequence having at least 85% identity with the amino acid residues 255 to 371 of Figure 8 (SEQ ID NO:8), said method comprising incorporating an isolated nucleic acid molecule encoding said amino acid residues into said vector in operatively linked relation with a promoter.

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67. A method for producing an activated truncated form of PDGF-D, comprising the steps of expressing an expression vector comprising a polynucleotide encoding a polypeptide as claimed in claim 25, claim 29 or claim 30 and supplying a proteolytic amount of at least one enzyme for processing the expressed polypeptide to generate the activated truncated form of PDGF-D.

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68. A method of inhibiting tissue remodeling during invasion of tumor cells into a normal population of cells, comprising administering to said mammal a PDGF-D inhibiting amount of a PDGF-C antagonist.

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aattgtggct gtggaactgt caactggagg tctgcacat gcaattcagg gaaaaccgtg 60
aaaaagtatc atgaggtatt acagtttgag cctggccaca tcaagaggag gggtagogct 120
aagaccatgg ctctagttga catccagttg gatcaccatg aacgatgtga ttgtatctgc 180
agctcaagac caccctgata agagaatgtg cacatccctt callaagcct gaaagaacca 240
ttagtttaag gagggtgaga taagagaccc ttctctacc agcaaccaga cttaactacta 300
gcctgcaatg caatgaacac aagtggttgc tgagtctcat ccttgctttg ttaatgccat 360

FIG.1

Asn	Cys	Gly	Cys	Gly	Thr	Val	Asn	Trp	Arg	Ser	Cys	Thr	Cys	Asn	Ser
1					5				10					15	
Gly	Lus	Thr	Val	Lys	Lys	Tyr	His	Glu	Val	Leu	Gln	Phe	Glu	Pro	Gly
			20					25					30		
His	Ile	Lys	Arg	Arg	Gly	Arg	Ala	Lys	Thr	Met	Ala	Leu	Val	Asp	Ile
		35					40					45			
Gln	Leu	Asp	His	His	Glu	Arg	Cys	Asp	Cys	Ile	Cys	Ser	Ser	Arg	Pro
	50					55					60				
Pro	Arg														
65															

FIG.2

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ggaagatttc caaccgcag cagcttcaga gaccaactgg aatctgtcac aagctctgtt 60
tcagggtatc cctataactc tccatcagta acggatccca ctctgattgc ggaatgtctg 120
gacaaaaaaaa ttgcagaatt tgatocagtg gaagatctgc tcaagtaact caatccagag 180
tcatggcaag aafatcttga gaatatgtat ctggacccc ctgggtatcg aggcaggta 240
taccatgacc ggaagtcaaa agttgacctg gataggctca atgatgatgc caagcgttac 300
agttgcactc ccaggaatta ctgggtcaat ataagagaag agctgaagtt ggccaatgag 360
gtcttcttcc caggttgctt cctcgtgcag cgtctgtggag gaaattgtgg ctgtggaaact 420
gtcaaacctgg agtctgtcac atgcaattca gggaaaaccg tgaaaaagta tcatgaggta 480
ttacagtttg agcctggcca catcaagagg aggggttagag ctaagaccat ggcctagtt 540
gacatccagt tggatcacca tgaacgatgc gattgtatct gcagctcaag accacctcga 600
taagagaatg tgcacatcct tacaatgaac ctgaagaac ctttagttta aggaggggtga 660
gataagagac ccttttctta ccagcaacc 690

FIG.3

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Gly	Arg	Phe	Pro	Thr	Arg	Ser	Ser	Phe	Arg	Asp	Gln	Leu	Glu	Ser	Val
1				5					10					15	
Thr	Ser	Ser	Val	Ser	Gly	Tyr	Pro	Tyr	Asn	Ser	Pro	Ser	Val	Thr	Asp
			20					25					30		
Pro	Thr	Leu	Ile	Ala	Asp	Ala	Leu	Asp	Lys	Lys	Ile	Ala	Glu	Phe	Asp
		35					40					45			
Thr	Val	Glu	Asp	Leu	Leu	Lys	Tyr	Phe	Asn	Pro	Glu	Ser	Trp	Gln	Glu
	50					55					60				
Asp	Leu	Glu	Asn	Met	Tyr	Leu	Asp	Thr	Pro	Arg	Tyr	Arg	Gly	Arg	Ser
65					70					75					80
Tyr	His	Asp	Arg	Lys	Ser	Lys	Val	Asl	Leu	Asp	Arg	Leu	Asn	Asp	Asp
				85					90					95	
Ala	Lys	Arg	Tyr	Ser	Cys	Thr	Pro	Arg	Asn	Tyr	Ser	Val	Asn	Ile	Arg
			100					105					110		
Glu	Glu	Leu	Lys	Leu	Ala	Asn	Val	Val	Phe	Phe	Pro	Arg	Cys	Leu	Leu
		115					120					125			
Val	Gln	Arg	Cys	Gly	Gly	Asn	Cys	Gly	Cys	Gly	Thr	Val	Lys	Leu	Glu
		130				135					140				
Ser	Cys	Thr	Cys	Asn	Ser	Gly	Lys	Thr	Val	Lys	Lus	Tyr	His	Glu	Val
145					150					155					160
Leu	Gln	Phe	Glu	Pro	Gly	His	Ile	Lys	Arg	Arg	Gly	Arg	Ala	Lys	Thr
				165					170					175	
Met	Ala	Leu	Val	Asp	Ile	Gln	Leu	Asp	His	His	Glu	Arg	Cys	Asp	Cys
			180					185					190		
Ile	Cys	Ser	Ser	Arg	Pro	Pro	Arg								
			195				200								

FIG.4

SUBSTITUTE SHEET (rule 26)

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ttglaccgaa gagatgagac catccaggig aaaggaaacg gctacgtlga gaglccatga 60
 ttcccgaaca gclaccccag gaacctgclc clgacatggc ggcttcaclc tcaggagaaat 120
 acacggatoc agclagtgtt lgacaalcag ttgggallag aggaagcaga aaatgatatt 180
 tglaggatag attttgtgga agttgaagat alalcgaaa ccagtaccat taitagaggaa 240
 cgalgggtgig gacacoaggga agltccctcc aaatlaaaa caagaacgaa ccaaatlaaa 300
 alcacallca aglccgaltga clactttgig gclaaacctg gaitcaagat ttattattct 360
 ttgcttggag atttccaacc cgcagcagct tcagagacca actgggaalc tgcacoagc 420
 tclatttcag ggglatccca taactctcca tcaglaacgg atcccacr cr gatttctfat 480
 gclctggaca aaaaaattgc agaattgat acagltggag atclgtlcaa glacttcaat 540
 ccagagltcal ggcaagaaga tcltgagaat atglatclgg acacccctcg gtattgggc 600
 aggtcatacc atgaccggaa glcaaaagtt gacctggata ggclcaatga lcatgccaa 660
 cgttacagtt gcactccag gaattacteg glcaatataa gagaagagct gaagltggc 720
 aatgtggctt tclttccag ttgcctcclc gtcagcagct gtggaggaaa ttgtggctgt 780
 ggaactgtca actggaggte clgcacatgc aatlcaggga aaaccgtgaa aaagtalcot 840
 gaggtattac aglttgagcc tggccacalc aagaggaggg gttagagcaa gacctggct 900
 clagtltgaa tccagtltga tccactgaa cgalgcgatt gtatctgcag clcaagacca 960
 cctcgataag agaattgtca catccttaca tlaagcctga aagaaccttt aglttaagga 1020
 gggigagata agagacctt ttclaccag caaccaaat tactactagc clgcaatga 1080
 atgaacacaa gtggltgtig agtclagcc ttgctttgtt aatgccatgg caagttagaa 1140
 ggtataltat caacttctat acctagaat ataggattgc atttaataat aglttttag 1200
 gttataltat cacaacacaa cacagaata tattcatgtc tatgtgtata tagatcaaat 1260
 gttttttttg gtatolataa ccaggtacac cagagcttac atatgtttga gtttagact 1320
 taaaatcctt tgcacaaata agggatggc aaatolatga aacatgtctt tagaaattt 1380
 agggatlaaa ttattttttt aattttgaaa cacaacacaa ttltgaa tct tgcctcttla 1440
 aagaagcat cltgtatatt aaaaatcaaa agatgaggct ttcltacta tacatcttag 1500
 ttgatttlaa aaaaaggaaa aagglttcca gagaagaggc caatacc taa gcatttttc 1560
 calgagaagc actgcatact tactatgtg gactgtlaa accgtctcc aaacacatgc 1620
 cataataata taagtgttt agaaatlaaa tcatgtgtt ttltatgcat ttgtctgagg 1680
 catccttatt catllaacac clatctcaaa aacttactta gaaggtttt taltatagtc 1740
 ctacaaaaga caatgtataa gclgtacag aattttgaat tgtttttct tgcacaaacc 1800
 ctccacaaaa gcaatcctt tcaagaatgg calggcatt clgtatgac ctltccagat 1860
 ggtgttcogt gaagatgtg ggtagttag aacttaaaaa gigaacattg aaacatcgac 1920
 glaactggaa accg 1934

FIG.5

SUBSTITUTE SHEET (rule 26)

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Leu	Tyr	Arg	Arg	Asp	Glu	Thr	Ile	Gln	Val	Lys	Gly	Asn	Gly	Tyr	Val	1	5	10	15
Gln	Ser	Pro	Arg	Phe	Pro	Asn	Ser	Tyr	Pro	Arg	Asn	Leu	Leu	Leu	Thr	20	25	30	
Trp	Arg	Leu	His	Ser	Gln	Glu	Asn	Thr	Arg	Ile	Gln	Leu	Val	Phe	Asp	35	40	45	
Asn	Gln	Phe	Gly	Leu	Glu	Glu	Ala	Glu	Asn	Asp	Ile	Cys	Arg	Tyr	Asp	50	55	60	
Phe	Val	Glu	Val	Glu	Asp	Ile	Ser	Glu	Thr	Ser	Thr	Ile	Ile	Arg	Gly	65	70	75	80
Arg	Trp	Cys	Gly	His	Lys	Glu	Val	Pro	Pro	Arg	Ile	Lys	Ser	Arg	Thr	85	90	95	
Asn	Gln	Ile	Lys	Ile	Thr	Phe	Lys	Ser	Asp	Asp	Tyr	Phe	Val	Ala	Lys	100	105	110	
Pro	Gly	Phe	Lys	Ile	Tyr	Tyr	Ser	Leu	Leu	Glu	Asp	Phe	Gln	Pro	Ala	115	120	125	
Ala	Ala	Ser	Glu	Thr	Asn	Trp	Glu	Ser	Val	Thr	Ser	Ser	Ile	Ser	Gly	130	135	140	
Val	Ser	Try	Asn	Ser	Pro	Ser	Val	The	Asp	Pro	Thr	Lei	Ile	Ala	Asp	145	150	155	160
Ala	Leu	Asp	Lys	Lys	Ile	Ala	Glu	Phe	Asp	The	Val	Glu	Asp	Leu	Leu	165	170	175	
Lys	Tyr	Phe	Asn	Pro	Glu	Ser	Trp	Gln	Glu	Asp	Leu	Glu	Asn	Met	Tyr	180	185	190	
Leu	Asp	Thr	Pro	Arg	Tyr	Arg	Gly	Arg	Ser	Tyr	His	Asp	Arg	Lys	Ser	195	200	205	
Lys	Val	Asp	Leu	Asp	Arg	Leu	Asn	Asp	Asp	Ala	Lys	Arg	Tyr	Ser	Cys	210	215	220	

FIG. 6A

SUBSTITUTE SHEET (rule 26)

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Thr	Pro	Arg	Asn	Tyr	Ser	Val	Asn	Ile	Arg	Glu	Glu	Leu	Lus	Leu	Ala
225					230					235					240
Asn	Val	Val	Phe	Phe	Pro	Arg	Cys	Leu	Leu	Val	Gln	Arg	Cys	Asn	Ser
			245						250					255	
Asn	Cys	Gly	Cys	Gly	Thr	Val	Asn	Trp	Arg	Ser	Cys	Thr	Cys	Asn	Ser
		260						265					270		
Gly	Lys	Thr	Val	Lys	Lys	Tyr	His	Glu	Val	Leu	Gln	Phe	Glu	Pro	Gly
		275					280					285			
His	Ile	Lys	Arg	Arg	Gly	Arg	Ala	Lys	Thr	Met	Ala	Leu	Val	Asp	Ile
	290					295					300				
Gln	Leu	Asp	His	His	Glu	Arg	Cys	Asp	Cys	Ile	Cus	Ser	Ser	Arg	Pro
305					310					315					320
Pro	Arg														

FIG. 6B

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cgctcggaaa gttcagcatg caggaagitt ggggagagct cggcgattag cacagcgacc 60
 cgggccagcg cagggcgagc gcaggcggcg agagcgagg gcggcgagg gtcgggccg 120
 ggagcagaac ccgcttttt cttggagcga cgcgtctct agtcgtgat cccaa 175
 atg cac cgg ctc atc ttt gtc tac act cta atc tgc gca aac ttt tgc 223
 Met His Arg Leu Ile Phe Val Tyr Thr Leu Ile Cys Ala Asn Phe Cys
 1 5 10 15
 agc tgt cgg gac act tct gca acc ccg cag agc gca tcc atc aaa gct 271
 Ser Cys Arg Asp The Ser Ala Thr Pro Gln Ser Ala Ser Ile Lus Ala
 20 25 30
 ttg cgc aac gcc aac ctc agg cga gat gag agc aat cac ctc aca gac 319
 Leu Arg Asn Ala Asn Leu Arg Arg Asp Glu Ser Asn His Leu Thr Asp
 35 40 45
 ttg tac cga aga gat gag acc atc cag gtg aaa gga aac ggc tac gtg 367
 Leu Tyr Arg Arg Asp Glu Thr Ile Gln Val Lys Gly Asn Gly Tyr Val
 50 55 60
 cag agt cct aga ttc ccg aac agc tac ccc agg aac ctg ctc ctg aca 415
 Gln Ser Pro Arg Phe Pro Asn Ser Tyr Pro Arg Asn Leu Leu Leu Thr
 65 70 75 80
 tgg cgg ctt cac tct cag gag aat aca cgg ala cat cta gtg ttt gac 463
 Trp Arg Leu His Ser Gln Glu Asn Thr Arg Ile Gln Leu Val Phe Asp
 85 90 95
 aat cag ttt gga tta gag gaa gca gaa aat gat atc tgt agg tat gat 511
 Asn Gln Phe Gly Leu Glu Glu Ala Glu Asn Asp Ile Cys Arg Tyr Asp
 100 105 110
 ttt gtg gaa gtt gaa gat ata tcc gaa acc agt acc att att aga gga 559
 Phe Val Glu Val Glu Asp Ile Ser Glu Thr Ser The Ile Ile Arg Gly
 115 120 125
 cga tgg tgt gga cac aag gaa gtt cct cca agg ata aaa tca aga acg 607
 Arg Trp Cys Gly His Lys Glu Val Pro Pro Arg Ile Lys Ser Arg Thr
 130 135 140
 aac caa att aaa atc aca ttc aag tcc gat gac tac ttt gtg gct aaa 655
 Asn Gln Ile Lys Ile Thr Phe Pys Ser Asp Asp Tyr Phe Val Ala Lys
 145 150 155 160

FIG. 7A

SUBSTITUTE SHEET (rule 26)

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cct gga ttc aag att tat tat tct ttg ctg gaa gat ttc caa ccc gca	703
Fro Gly Phe Lys Ile Tyr Tyr Ser Leu Leu Glu Asp Phe Gln Pro Ala	
165 170 175	
gca gct tta gag acc aac tgg gaa tct glc aca agc tct att tca ggg	751
Ala Ala Ser Glu Thr Asn Trp Glu Ser Val Thr Ser Ser Ile Ser Gly	
180 185 190	
gta tcc tat aac tct cca tca gla act gat ccc act ctg att gag gat	799
Val Ser Tyr Asn Ser Pro Ser Val Thr Asp Pro Thr Leu Ile Ala Asp	
195 200 205	
gct ctg gac aaa aaa att gca gaa ttt gat aca gtg gaa gat ctg ctc	847
Ala Leu Asp Lys Lys Ile Ala Glu Phe Asp Thr Val Glu Asp Leu Lei	
210 215 220	
aag tac ttc aat cca gag tca tgg caa gaa gat ctt gag aat atg tat	895
Lys thr Phe Asn Pro Glu Ser Trp Gln Glu Asp Leu Flu Asn Met Tyr	
225 230 235 240	
ctg gac acc cct cgg tat cga ggc agg tca tac cat gac cgg aag tca	943
Leu Asp Thr Pro Arg Tyr Arg Gly Arg Ser Tyr His Asp Arg Lys Ser	
245 250 255	
aaa gtt gac ctg gat agg ctc aat gat gat gcc aag cgt tac agt tgc	991
Lys Val Asl Leu Asp Arg Leu Asn Asp Asp Ala Lus Arg Tyr Ser Cys	
260 265 270	
act ccc agg aat tac tcg gtc aat ata aga gaa gag ctg aag ttg gcc	1039
Thr Pro Arg Asn Try Ser Val Asn IIs Arg Glu Glu Leu Lys Leu Ala	
275 280 285	
aat glg gtc ttc ttt cca cgt tgc ctc ctc glg cag cgc tgt gga gga	1087
Asn Val Val Phe Phe Pro Arg Cys Leu Leu Val Gln Arg Cys Gly Gly	
290 295 300	
aat tgt ggc tgt gga act gtc aac tgg agg tcc tgc aca tgc aat tca	1135
Asn Cys Gly Cys Gly Thr Val Asn Trg Arg Ser Cys Thr Cys Asn Ser	
305 310 315 320	
ggg aaa acc glg aaa aag tat cat gag gla tta cag ttt gag cct ggc	1183
Gly Lys Thr Val Lys Lys Tyr His Glu Val Leu Gln Phe Glu Pro Gly	
325 330 335	

FIG. 7B

SUBSTITUTE SHEET (rule 26)

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cac atc aag agg agg ggt aga gct aag acc atg gct cta gtt gac atc 1231
 His Ile Lys Arg Arg Gly Arg Ala Lys Thr Met Ala Leu Val Asp Ile
 340 345 350
 cag ttg gat cac cat gaa cga tgc gat tgt atc tgc agc tca aga cca 1279
 Gln Leu Asp His His Glu Arg Cys Asp Cys Ile Cys Ser Ser Arg Pro
 355 360 365
 cct cga taagagaatg tgcacatcct tacatttaagc ctgaagaac ctttagttta 1335
 Pro Arg
 370

aggggggtga gataagagac ccttttccct ccagcaacca aacttaactac tagcctgcaa 1396
 tgcattgaac acaagtgggt gctgagctc agccttgctt tgltaalgcc atggcaagta 1455
 gaaaggtata tcatcaactt ctatacctaa gaatalagga ttgcatttaa taatagtggt 1515
 tgaggltata tatgcacaaa cacacacaga aatatattca tglctatgtg tatatagatc 1575
 aatgttttt ttgggtatat ataaccagggt acaccagagc ttacatagt ttgagltaga 1635
 ctcttaaaat ccttggccaa aataagggtt ggtaaatat atgaacatg tctttagaaa 1695
 atttaggaga taattttatt tttaatttt gaaacacaaa acaatttga atcttgctct 1755
 cttaagagaa gcatcttga taltaaaat caaagatga ggccttctta catatacatc 1815
 ttagttagt attaaaaag gaaaagggt tccagagaaa aggccaatac ctaagcattt 1875
 ttccatgag aagcactgca tacttaccta tgtggactgt aataacctgt ctccaaaacc 1935
 atgccaat aatataagtg ctttagaat taatcattg tgtttttat gcatttgct 1995
 gaggcacct tattcattta acacctatc caaaaactta cttagaagggt tttttattat 2055
 agtctacaa aagacaatgt ataagctga acagaatttt gaattgttt tctttgcaa 2115
 accctccac aaagcaaat cctttcaaga atggcatggg catctgtat gaaccttcc 2175
 agatgggtt cogtgaaga tgtgggtagt tgagaactta aaagtgaac attgaacat 2235
 cgacgtact ggaaccg???????2253

FIG. 7C

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Met	His	Arg	Leu	Ile	Phe	Val	Tyr	Thr	Leu	Ile	Cys	Ala	Asn	Phe	
1				5					10					15	
Cys	Ser	Cys	Arg	Asp	Thr	Ser	Ala	Thr	Pro	Gln	Ser	Ala	Ser	Ile	Lys
				20					25					30	
Ala	Leu	Arg	Asn	Ala	Asn	Leu	Arg	Arg	Asp	Glu	Ser	Asn	His	Leu	Thr
				35					40					45	
Asp	Leu	Tyr	Arg	Arg	Asp	Glu	Thr	Ile	Gln	Val	Lys	Gly	Asn	Gly	Tyr
		50					55					60			
Val	Gln	Ser	Pro	Arg	Phe	Pro	Asn	Ser	Tyr	Pro	Arg	Asn	Leu	Leu	Leu
	65						70				75				
Thr	Trp	Arg	Leu	His	Ser	Gln	Glu	Asn	Thr	Arg	Ile	Gln	Leu	Val	Phe
80					85					90					95
Asp	Asn	Gln	Phe	Gly	Leu	Glu	Glu	Ala	Glu	Asn	Asp	Ile	Cys	Arg	Tyr
				100					105					110	
Asp	Phe	Val	Glu	Val	Glu	Asp	Ile	Ser	Glu	Thr	Ser	Thr	Ile	Ile	Arg
				115					120				125		
Gly	Arg	Trp	Cys	Gly	His	Lys	Glu	Val	Pro	Pro	Arg	Ile	Lys	Ser	Arg
		130					135						140		
Thr	Asn	Gln	Ile	Lys	Ile	Thr	Phe	Lys	Ser	Asp	Asp	Tyr	Phe	Val	Ala
	145						150				155				
Lys	Pro	Gly	Phe	Lys	Ile	Tyr	Tyr	Ser	Leu	Leu	Glu	Asp	Phe	Gln	Pro
160					165					170					175
Ala	Ala	Ala	Ser	Glu	Thr	Asn	Trp	Glu	Ser	Val	Thr	Ser	Ser	Ile	Ser
				180					185					190	
Gly	Val	Ser	Tyr	Asn	Ser	pro	Ser	Val	Thr	Asp	Pro	Thr	Leu	Ile	Ala
			195						200				205		
Asp	Ala	Leu	Asp	Lys	Lys	Ile	Ala	Glu	Phe	Asp	Thr	Val	Glu	Asp	Leu
		210					215						220		
Leu	Lys	Thr	Phe	Asn	Pro	Glu	Ser	trp	Gln	Glu	Asp	Leu	Glu	Asn	Met
		225					230						235		

FIG. 8A

SUBSTITUTE SHEET (rule 26)

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Tyr	Leu	Ash	Thr	Pro	Arg	Tyr	Arg	Gly	Arg	Ser	Tyr	His	Asp	Arg	Lys
240					245					250					255
Ser	Lys	Val	Asp	Leu	Asp	Arg	Leu	Asn	Asp	Asp	Ala	Lys	Arg	Tyr	Ser
			260						265						270
Cys	Thr	Pro	Arg	Asn	Tyr	Ser	Val	Asn	Ile	Arg	Glu	Glu	Leu	Lys	Leu
			275						280						285
Ala	Asn	Val	Val	Phe	Phe	Pro	Arg	Cys	Leu	Leu	Val	Gln	Arg	Cys	Gly
		290					295						300		
Gly	Asn	Cys	Cly	Cys	Gly	Thr	Val	Asn	Trp	Arg	Ser	Cys	Thr	Cus	Asn
	305						310					315			
Ser	Gly	Lys	Thr	Val	Lys	Lys	Tyr	His	Glu	Val	Leu	Gln	Phe	Glu	Pro
320					325					330					335
Glu	His	Ile	Lys	Arg	Arg	Gly	Arg	Ala	Lys	Thr	Met	Ala	Leu	Val	Asp
			340						345						350
Ila	Glm	Leu	Asp	His	His	Gln	Arg	Cys	Asp	Cys	Ile	Cys	Ser	Ser	Arg
			355					360					365		
Pro	Pro	Arg													
		370													

FIG. 8B

SUBSTITUTE SHEET (rule 26)

FIG. 9A

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VEGF 165	MN	L	L	S	W	V	H	W	S	L	A	L	L	Y	L	H	H	A	K	W	S	Q	A	A	P	M	A	E	G	G	G	Q	N	H	H	E	V	45					
PIGF-2	MP	V	M	R	L	F	P	C	F	L	Q	L	A	G	L	A	L	P	A	V	P	P	Q	Q	W	A	L	S	A	G	N	G	S	S	E	V	E	V	40				
VEGF-B167	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	15							
Pox Orf VEGF	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24								
VEGF-C	Y	K	C	O	L	R	K	G	G	W	Q	H	N	R	E	Q	A	N	L	S	R	T	E	E	T	Y	K	F	A	A	H	Y	N	T	E	L	-	119					
VEGF-D	D	W	K	L	W	R	C	R	L	K	L	K	S	L	A	S	M	D	S	R	S	A	S	H	R	S	T	R	F	A	A	T	F	Y	D	T	E	Y	-	104			
PCGF-A	L	L	E	I	D	S	V	G	S	E	D	S	L	D	T	S	L	R	A	H	G	V	H	-	-	A	I	K	H	V	P	E	K	R	P	L	P	I	R	54			
PCGF-B	D	P	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	89						
hPCGF-D	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1212							
VEGF 165	V	K	F	M	D	V	Y	Q	R	S	Y	C	H	P	I	E	T	L	V	D	I	F	C	E	Y	P	D	H	I	E	Y	I	F	K	-	-	P	S	C	V	76		
PIGF-2	V	P	F	Q	E	V	W	G	R	S	Y	C	R	A	L	E	R	L	V	D	V	S	E	Y	P	S	E	V	E	E	M	F	S	-	-	-	-	P	S	C	V	78	
VEGF-B167	V	S	W	I	D	V	Y	T	R	A	T	C	Q	F	R	F	V	V	P	L	T	V	E	L	M	G	T	V	S	K	Q	L	V	-	-	-	-	P	S	C	V	73	
Pox Orf VEGF	K	G	W	S	E	V	L	K	G	S	E	C	K	P	R	P	I	V	V	P	V	S	E	T	H	P	E	L	T	S	Q	R	F	N	-	-	-	-	P	P	C	V	62
VEGF-C	K	S	I	S	N	E	W	R	K	T	Q	C	M	P	R	E	V	C	I	D	V	G	K	E	F	G	V	A	T	N	T	F	F	K	-	-	-	-	P	P	C	V	157
VEGF-D	K	V	I	D	E	E	W	Q	R	T	Q	C	S	P	R	E	T	C	V	E	V	A	S	E	L	G	K	T	I	N	T	F	F	K	-	-	-	-	P	P	C	V	142
PCGF-A	K	R	S	T	E	E	A	V	P	A	V	C	K	T	R	T	V	I	Y	E	I	P	R	S	Q	V	D	P	T	S	A	N	F	L	I	W	P	P	C	V	124		
PCGF-B	L	T	T	A	E	P	A	M	I	A	E	C	K	T	R	T	E	V	F	E	I	S	R	R	L	I	D	R	I	N	A	N	F	L	V	W	P	P	C	V	125		
hPCGF-D	D	K	L	N	D	D	A	K	R	Y	S	C	T	P	R	N	Y	S	V	N	I	-	R	E	E	L	K	L	A	N	V	V	F	F	-	-	-	-	P	R	C	L	249

FIG. 9B

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VEGF 165	P L M R C G G	CCND	EGLE	C V P T	E E S N	I T M	Q I M R	I K	--	P R	112
PIGF-2	S L L R C T G	CCGD	EDLH	C V P V	E T A N	V T M	Q L K I	A	--	S G	112
VEGF-B167	T V Q R C G G	CCPD	DGLE	C V P T	T G Q H	V R M	Q I L M	I R Y	--	P S	108
Pox Orf VEGF	T L M R C G G	CCND	ESLE	C V P T	E E V N	V S M E	L L G A	S G S G	S N	99	
VEGF-C	S V Y R C G G	CCNS	EGLQ	C M N T	S T S Y	L S K I	I L F E	I T V	--	P L	192
VEGF-D	N V F R C G G	CCNE	EGVM	C M N T	S T S Y	I S K Q	L F E I	S V	--	P L	177
PCGF-A	E V K R C T G	CCNT	SSVK	K C Q P S R	V H H R	S V K V	A K V E	Y V R K K	161		
PCGF-B	E V Q R C S G	CCNN	RNVQ	C R P T	C V Q L	R P V Q	V R K I	E I V R K K	162		
hPCGF-D	L V Q R C G G	CCG	T V N W R S C	T C N S	G K T V	K Y H E	V L Q F E P G H	289			
VEGF 165	Q G Q	--	H I G E M S	F L Q H N K	--	C E C R P	K K	--	--	--	135
PIGF-2	D R F	--	S Y V E L T	P S Q E V R	--	C E C R P	L R E	--	--	K M K	140
VEGF-B167	S Q L	--	--	G E M S L E E H S Q	--	C E C K P	K K K	--	--	D S A V	133
Pox Orf VEGF	G M Q	--	--	R L S F V E H K K	--	C D C R P	R F T	--	--	T T	121
VEGF-C	S O G	--	--	P K P V T I S F A N H T S	--	C R C M S	K L D	--	--	V Y K Q V H	222
VEGF-D	Y S V	--	--	P E L V P V K I A N H T G	--	C K C L P	T G P I	--	--	R H P Y	205
PCGF-A	P K L	--	--	K E V Q V R L E E H L E	--	C A C A T I	S L N P D Y	R E E D T	193		
PCGF-B	P I F	--	--	K K A T V T L E D E L A	--	C K C E T V A A A R P V T	R S P G	194			
hPCGF-D	I K R R G R A K T M A L V D I Q L D H H E R	--	--	--	--	C D C I C S S R P P R	322				

FIG. 9C

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VEGF 165	DRA	Q	E	N	P	C	G	P	C	S	S	E	R	R	K	K	L	F	V	Q	D	P	Q	T	C	K	C	S	C	K	N	I	D	S	-	R	C	173		
PICF-2	RRR	P	K	G	R	G	K	R	R	E	N	Q	R	P	T	D	C	H	L	C	G	L	A	V	P	R											170			
VEGF-B167	KPD	S	P	R	P	L	C	P	R	C	T	Q	H	H	Q	R	P	D	P	R	T	-	-	-	-	C	R	C	R	R	R	S	P	L	R	C	169			
Pox Orf VEGF	PPT	T	T	R	P	P	R	R	R																										133					
VEGF-C	SI	I	R	R	S	L	P	A	T	-	L	P	Q	C	Q	A	A	N	I	C	P	T	N	Y	M	N	N	H	I	C	R	C	L	A	Q	E	D	261		
VEGF-D	SI	I	R	R	S	I	O	I	P	E	E	D	E	C	P	H	S	K	K	L	C	P	I	D	M	L	W	D	N	T	K	C	K	C	V	L	O	D	E	245
PCGF-A	GR	P	I	R	E	S	G	K	K	R	K	L	K	P	T																					211				
PCGF-B	GS	Q	E	Q	R	A	K	T	P	Q	T	R	V	T	I	R	T	V	R	V	R	R	P	P	K	G	K	H	R	K	F	K	H	T	H	D	K	T	A	234
hPDGF-D																																				322				
VEGF 165	KAR	Q	L	E	L	N	E	R	T	C	R	C	D	K	P	R																					152			
PICF-2																																				170				
VEGF-B167	QGR	G	L	E	L	N	P	D	T	C	R	C	R	K	L	R																					188			
Pox Orf VEGF																																				133				
VEGF-C	FM	F	S	S	D	A	G	D	D	S	I	D	G	F	E	I	C	G	P	N	K	E	L	D	E	E	T	C	Q	C	V	C	R	A	G	L	R	P	301	
VEGF-D	-	T	P	L	F	G	T	E	D	H	S	Y	L	O	E	P	T	L	C	G	P	H	M	T	F	D	E	D	R	-	-	-	-	-	-	-	-	-	274	
PDGF-A																																				211				
PDGF-B	LKE	T	L	G	A																																241			
hPDGF-D																																				322				

FIG. 9D

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VEGF 165	192
PIGF-2	170
VEGF-B167	188
Pox Orf VEGF	133
VEGF-C	341
VEGF-D	295
PCGF-A	211
PCGF-B	241
hPDGF-D	322
VEGF 165	192
PIGF-2	170
VEGF-B167	188
Pox Orf VEGF	133
VEGF-C	381
VEGF-D	317
PCGF-A	211
PCGF-B	241
hPDGF-D	322

A S C G P H K E L D R N S C I Q C V C K N K L F P S Q C G A N R E F D E N T C Q C
 - - - - - C E C V C K A P C P G D L I O H P E N - - - - - C S C

V C K R T C P R N O P L N P G K C A C E C T E S P Q K C L L K G K K F H H Q T C
 F E C K E S L E S C C O K H K I - - - - - F H P D T C

FIG. 9E

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VEGF 165	192	
PIGF-2	170	
VEGF-B167	188	
Pox Orf VEGF	133	
VEGF-C	419	
VEGF-D	356	
PCGF-A	211	
PCGF-B	241	
hPDGF-D	322	
VEGF 165	192	
PIGF-2	170	
VEGF-B167	188	
Pox Orf VEGF	133	
VEGF-C	419	
VEGF-D	352	
PCGF-A	211	
PCGF-B	241	
hPDGF-D	322	

SUBSTITUTE SHEET (rule 26)

FIG. 9F

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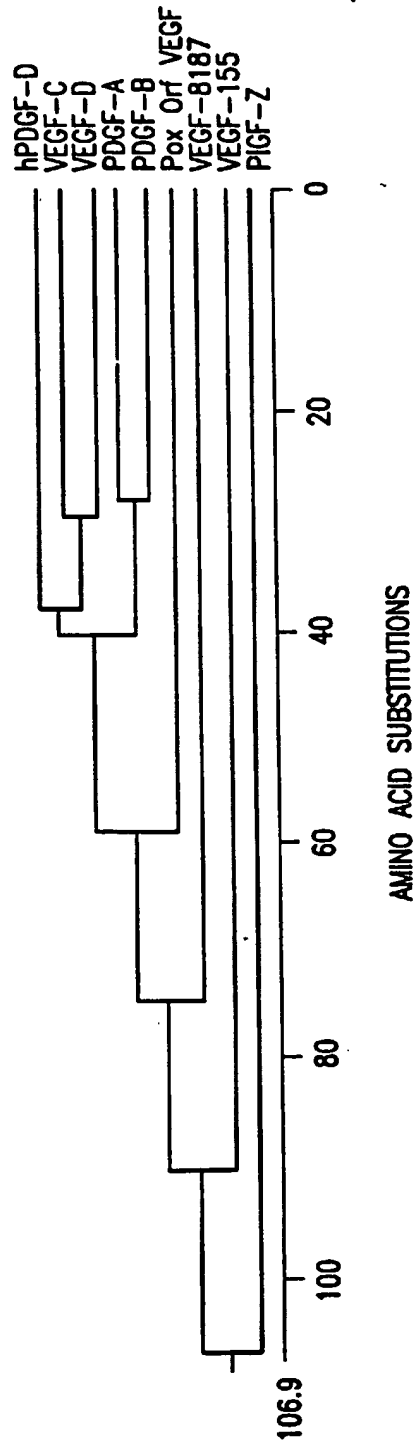


FIG.10

SUBSTITUTE SHEET (rule 26)

[illegible]

FIG. 11

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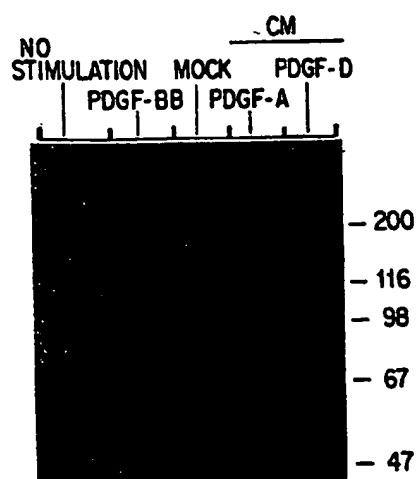


FIG. 12

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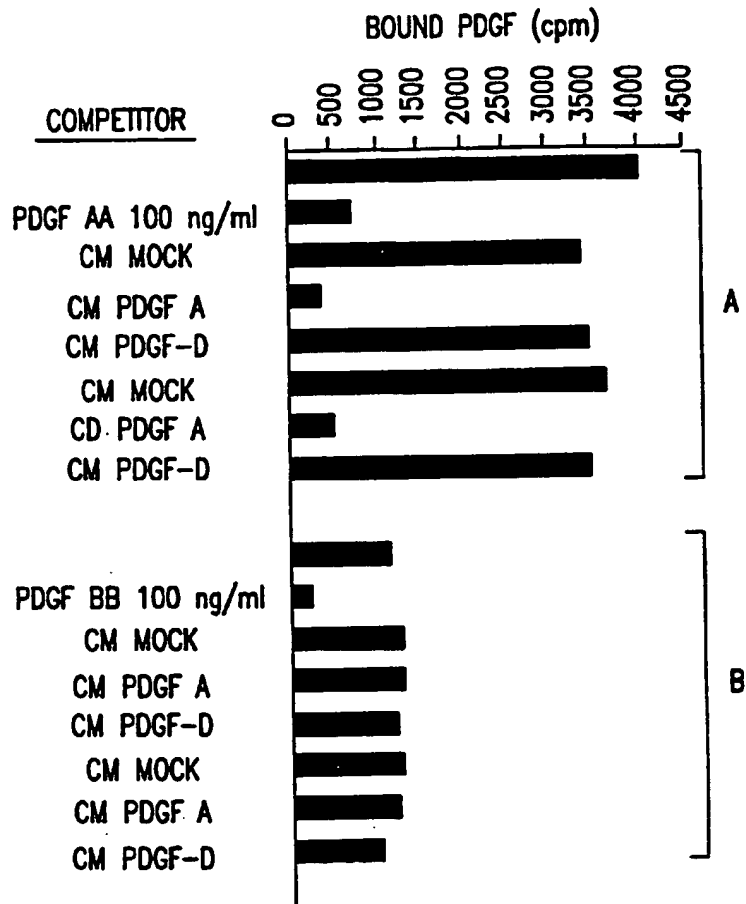


FIG. 13

SEQUENCE LISTING

<110> ERIKSSON, Ulf
AASE, Karin
LEE, Xuri
PONTUN, Annica
UUTELA, Marko
ALITALO, Kari
OESTMAN, Arne
HELDIN, Carl-Henrik

<120> PLATELET-DERIVED GROWTH FACTOR D, DNA CODING
THEREFOR, AND USES THEREOF

<130> Ulf Eriksson et al 1064-44833PC

<140>
<141>

<150> 60/107,852
<151> 1998-11-10

<150> 60/113,997
<151> 1999-12-28

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<151> 1999-08-26

<150> 60/157,108
<151> 1999-10-04

<150> 60/157,756
<151> 1999-10-05

<160> 31

<170> PatentIn Ver. 2.0

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<213> Homo sapiens

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aaaaagtatc atgaggtatt acagtttgag cctggccaca tcaagaggag gggtagagct 120

aagaccatgg ctctagttga catccagttg gatcaccatg aacgatgtga ttgtatctgc 180
 agctcaagac cacctcgata agagaatgtg cacatcctta cattaagcct gaaagaacca 240
 ttagtttaag gagggtgaga taagagaccc ttttctacc agcaaccaga cttactacta 300
 gcctgcaatg caatgaacac aagtggttgc tgagtctcag ccttgctttg ttaatgccat 360

<210> 2

<211> 66

<212> PRT

<213> Homo sapiens

<400> 2

Asn	Cys	Gly	Cys	Gly	Thr	Val	Asn	Trp	Arg	Ser	Cys	Thr	Cys	Asn	Ser
1				5					10					15	

Gly	Lys	Thr	Val	Lys	Lys	Tyr	His	Glu	Val	Leu	Gln	Phe	Glu	Pro	Gly
			20					25					30		

His	Ile	Lys	Arg	Arg	Gly	Arg	Ala	Lys	Thr	Met	Ala	Leu	Val	Asp	Ile
		35					40					45			

Gln	Leu	Asp	His	His	Glu	Arg	Cys	Asp	Cys	Ile	Cys	Ser	Ser	Arg	Pro
	50					55					60				

Pro Arg

65

<210> 3

<211> 690

<212> DNA

<213> Homo sapiens

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 tcagggtatc cctataactc tccatcagta acggatccca ctctgattgc ggatgctctg 120
 gacaaaaaaa ttgcagaatt tgatacagtg gaagatctgc tcaagtactt caatccagag 180
 tcatggcaag aagatcttga gaatatgtat ctggacaccc ctcggtatcg aggcagggtca 240
 taccatgacc ggaagtcaaa agttgacctg gataggctca atgatgatgc caagcggtac 300
 agttgcactc ccaggaatta ctcggtcaat ataagagaag agctgaagtt ggccaatgtg 360

gtcttctttc cacgttgccct cctcgtgcag cgctgtggag gaaattgtgg ctgtggaact 420
 gtcaaactgg agtcctgcac atgcaattca gggaaaaccg tgaaaaagta tcatgaggta 480
 ttacagtttg agcctggcca catcaagagg aggggtagag ctaagacat ggctctagtt 540
 gacatccagt tggatcacca tgaacgatgc gattgtatct gcagctcaag accacctcga 600
 taagagaatg tgcacatcct tacattaagc ctgaaagaac ctttagttta aggaggggtga 660
 gataagagac ccttttccta ccagcaaccc 690

<210> 4

<211> 200

<212> PRT

<213> Homo sapiens

<400> 4

Gly	Arg	Phe	Pro	Thr	Arg	Ser	Ser	Phe	Arg	Asp	Gln	Leu	Glu	Ser	Val
1				5					10					15	
Thr	Ser	Ser	Val	Ser	Gly	Tyr	Pro	Tyr	Asn	Ser	Pro	Ser	Val	Thr	Asp
			20						25					30	
Pro	Thr	Leu	Ile	Ala	Asp	Ala	Leu	Asp	Lys	Lys	Ile	Ala	Glu	Phe	Asp
		35						40					45		
Thr	Val	Glu	Asp	Leu	Leu	Lys	Tyr	Phe	Asn	Pro	Glu	Ser	Trp	Gln	Glu
		50					55						60		
Asp	Leu	Glu	Asn	Met	Tyr	Leu	Asp	Thr	Pro	Arg	Tyr	Arg	Gly	Arg	Ser
	65					70					75				80
Tyr	His	Asp	Arg	Lys	Ser	Lys	Val	Asp	Leu	Asp	Arg	Leu	Asn	Asp	Asp
				85						90					95
Ala	Lys	Arg	Tyr	Ser	Cys	Thr	Pro	Arg	Asn	Tyr	Ser	Val	Asn	Ile	Arg
				100					105					110	
Glu	Glu	Leu	Lys	Leu	Ala	Asn	Val	Val	Phe	Phe	Pro	Arg	Cys	Leu	Leu
		115						120					125		
Val	Gln	Arg	Cys	Gly	Gly	Asn	Cys	Gly	Cys	Gly	Thr	Val	Lys	Leu	Glu
		130					135					140			
Ser	Cys	Thr	Cys	Asn	Ser	Gly	Lys	Thr	Val	Lys	Lys	Tyr	His	Glu	Val

145 150 155 160
 Leu Gln Phe Glu Pro Gly His Ile Lys Arg Arg Gly Arg Ala Lys Thr
 165 170 175
 Met Ala Leu Val Asp Ile Gln Leu Asp His His Glu Arg Cys Asp Cys
 180 185 190
 Ile Cys Ser Ser Arg Pro Pro Arg
 195 200

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 <211> 1934
 <212> DNA
 <213> Homo sapiens

 <220>
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 <222> (1)..(966)

 <400> 5
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 Leu Tyr Arg Arg Asp Glu Thr Ile Gln Val Lys Gly Asn Gly Tyr Val
 1 5 10 15

 cag agt cct aga ttc ccg aac agc tac ccc agg aac ctg ctc ctg aca 96
 Gln Ser Pro Arg Phe Pro Asn Ser Tyr Pro Arg Asn Leu Leu Leu Thr
 20 25 30

 tgg cgg ctt cac tct cag gag aat aca cgg ata cag cta gtg ttt gac 144
 Trp Arg Leu His Ser Gln Glu Asn Thr Arg Ile Gln Leu Val Phe Asp
 35 40 45

 aat cag ttt gga tta gag gaa gca gaa aat gat atc tgt agg tat gat 192
 Asn Gln Phe Gly Leu Glu Glu Ala Glu Asn Asp Ile Cys Arg Tyr Asp
 50 55 60

 ttt gtg gaa gtt gaa gat ata tcc gaa acc agt acc att att aga gga 240
 Phe Val Glu Val Glu Asp Ile Ser Glu Thr Ser Thr Ile Ile Arg Gly
 65 70 75 80

 cga tgg tgt gga cac aag gaa gtt cct cca agg ata aaa tca aga acg 288
 Arg Trp Cys Gly His Lys Glu Val Pro Pro Arg Ile Lys Ser Arg Thr
 85 90 95

 aac caa att aaa atc aca ttc aag tcc gat gac tac ttt gtg gct aaa 336
 Asn Gln Ile Lys Ile Thr Phe Lys Ser Asp Asp Tyr Phe Val Ala Lys

100	105	110	
cct gga ttc aag att tat tat tct ttg ctg gaa gat ttc caa ccc gca			384
Pro Gly Phe Lys Ile Tyr Tyr Ser Leu Leu Glu Asp Phe Gln Pro Ala			
115	120	125	
gca gct tca gag acc aac tgg gaa tct gtc aca agc tct att tca ggg			432
Ala Ala Ser Glu Thr Asn Trp Glu Ser Val Thr Ser Ser Ile Ser Gly			
130	135	140	
gta tcc tat aac tct cca tca gta acg gat ccc act ctg att gcg gat			480
Val Ser Tyr Asn Ser Pro Ser Val Thr Asp Pro Thr Leu Ile Ala Asp			
145	150	155	160
gct ctg gac aaa aaa att gca gaa ttt gat aca gtg gaa gat ctg ctc			528
Ala Leu Asp Lys Lys Ile Ala Glu Phe Asp Thr Val Glu Asp Leu Leu			
165	170	175	
aag tac ttc aat cca gag tca tgg caa gaa gat ctt gag aat atg tat			576
Lys Tyr Phe Asn Pro Glu Ser Trp Gln Glu Asp Leu Glu Asn Met Tyr			
180	185	190	
ctg gac acc cct cgg tat cga ggc agg tca tac cat gac cgg aag tca			624
Leu Asp Thr Pro Arg Tyr Arg Gly Arg Ser Tyr His Asp Arg Lys Ser			
195	200	205	
aaa gtt gac ctg gat agg ctc aat gat gat gcc aag cgt tac agt tgc			672
Lys Val Asp Leu Asp Arg Leu Asn Asp Asp Ala Lys Arg Tyr Ser Cys			
210	215	220	
act ccc agg aat tac tcg gtc aat ata aga gaa gag ctg aag ttg gcc			720
Thr Pro Arg Asn Tyr Ser Val Asn Ile Arg Glu Glu Leu Lys Leu Ala			
225	230	235	240
aat gtg gtc ttc ttt cca cgt tgc ctc ctc gtg cag cgc tgt gga gga			768
Asn Val Val Phe Phe Pro Arg Cys Leu Leu Val Gln Arg Cys Gly Gly			
245	250	255	
aat tgt ggc tgt gga act gtc aac tgg agg tcc tgc aca tgc aat tca			816
Asn Cys Gly Cys Gly Thr Val Asn Trp Arg Ser Cys Thr Cys Asn Ser			
260	265	270	
ggg aaa acc gtg aaa aag tat cat gag gta tta cag ttt gag cct ggc			864
Gly Lys Thr Val Lys Lys Tyr His Glu Val Leu Gln Phe Glu Pro Gly			
275	280	285	
cac atc aag agg agg ggt aga gct aag acc atg gct cta gtt gac atc			912
His Ile Lys Arg Arg Gly Arg Ala Lys Thr Met Ala Leu Val Asp Ile			

290	295	300	
cag ttg gat cac cat gaa cga tgc gat tgt atc tgc agc tca aga cca			960
Gln Leu Asp His His Glu Arg Cys Asp Cys Ile Cys Ser Ser Arg Pro			
305	310	315	320
cct cga taagagaatg tgcacatcct tacattaagc ctgaaagaac ctttagttta			1016
Pro Arg			
aggaggggtga gataagagac ccttttccta ccagcaacca aacttactac tagcctgcaa			1076
tgcaatgaac acaagtgggt gctgagtctc agccttgctt tgtaaatgcc atggcaagta			1136
gaaaggtata tcatcaactt ctatacctaa gaatatagga ttgcatttaa taatagtgtt			1196
tgaggttata tatgcacaaa cacacacaga aatatattca tgtctatgtg tatatagatc			1256
aaatgttttt ttggtatat ataaccaggt acaccagagc ttacatatgt ttgagttaga			1316
ctcttaaaat cctttgccaa aataagggat ggtcaaatat atgaaacatg tctttagaaa			1376
atttaggaga taaatttatt tttaaatttt gaaacacaaa acaattttga atcttgctct			1436
cttaaagaaa gcatcttgta tattaataat caaaagatga ggctttctta catatacatc			1496
ttagttgatt attaaaaaag gaaaaagggt tccagagaaa aggccaatac ctaagcattt			1556
tttccatgag aagcactgca tacttaccta tgtggactgt aataacctgt ctccaaaacc			1616
atgccataat aatataagtg ctttagaaat taaatcattg tgttttttat gcattttgct			1676
gaggcatcct tattcattta acacctatct caaaaactta cttagaagggt tttttattat			1736
agtcctacaa aagacaatgt ataagctgta acagaatttt gaattgtttt tctttgcaa			1796
accctccac aaaagcaaat cctttcaaga atggcatggg cattctgtat gaacctttcc			1856
agatgggtgt cagtgaaga tgtgggtagt tgagaactta aaaagtgaac attgaaacat			1916
cgacgtaact ggaaaccg			1934

<210> 6

<211> 322

<212> PRT

<213> Homo sapiens

<400> 6

Leu Tyr Arg Arg Asp Glu Thr Ile Gln Val Lys Gly Asn Gly Tyr Val
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 Gln Ser Pro Arg Phe Pro Asn Ser Tyr Pro Arg Asn Leu Leu Leu Thr
 20 25 30
 Trp Arg Leu His Ser Gln Glu Asn Thr Arg Ile Gln Leu Val Phe Asp
 35 40 45
 Asn Gln Phe Gly Leu Glu Glu Ala Glu Asn Asp Ile Cys Arg Tyr Asp
 50 55 60
 Phe Val Glu Val Glu Asp Ile Ser Glu Thr Ser Thr Ile Ile Arg Gly
 65 70 75 80
 Arg Trp Cys Gly His Lys Glu Val Pro Pro Arg Ile Lys Ser Arg Thr
 85 90 95
 Asn Gln Ile Lys Ile Thr Phe Lys Ser Asp Asp Tyr Phe Val Ala Lys
 100 105 110
 Pro Gly Phe Lys Ile Tyr Tyr Ser Leu Leu Glu Asp Phe Gln Pro Ala
 115 120 125
 Ala Ala Ser Glu Thr Asn Trp Glu Ser Val Thr Ser Ser Ile Ser Gly
 130 135 140
 Val Ser Tyr Asn Ser Pro Ser Val Thr Asp Pro Thr Leu Ile Ala Asp
 145 150 155 160
 Ala Leu Asp Lys Lys Ile Ala Glu Phe Asp Thr Val Glu Asp Leu Leu
 165 170 175
 Lys Tyr Phe Asn Pro Glu Ser Trp Gln Glu Asp Leu Glu Asn Met Tyr
 180 185 190
 Leu Asp Thr Pro Arg Tyr Arg Gly Arg Ser Tyr His Asp Arg Lys Ser
 195 200 205
 Lys Val Asp Leu Asp Arg Leu Asn Asp Asp Ala Lys Arg Tyr Ser Cys
 210 215 220
 Thr Pro Arg Asn Tyr Ser Val Asn Ile Arg Glu Glu Leu Lys Leu Ala
 225 230 235 240
 Asn Val Val Phe Phe Pro Arg Cys Leu Leu Val Gln Arg Cys Gly Gly
 245 250 255

Asn Cys Gly Cys Gly Thr Val Asn Trp Arg Ser Cys Thr Cys Asn Ser
 260 265 270

Gly Lys Thr Val Lys Lys Tyr His Glu Val Leu Gln Phe Glu Pro Gly
 275 280 285

His Ile Lys Arg Arg Gly Arg Ala Lys Thr Met Ala Leu Val Asp Ile
 290 295 300

Gln Leu Asp His His Glu Arg Cys Asp Cys Ile Cys Ser Ser Arg Pro
 305 310 315 320

Pro Arg

<210> 7

<211> 2253

<212> DNA

<213> Homo sapiens

<220>

<221> CDS

<222> (176)..(1288)

<400> 7

cgctcggaaa gttcagcatg caggaagttt ggggagagct cggcgattag cacagcgacc 60

cgggccagcg cagggcgagc gcaggcgcg agagcgagg gcggcgcggc gtcggtcccg 120

ggagcagaac ccggcttttt cttggagcga cgctgtctct agtcgctgat cccaa atg 178
 Met
 1

cac cgg ctc atc ttt gtc tac act cta atc tgc gca aac ttt tgc agc 226
 His Arg Leu Ile Phe Val Tyr Thr Leu Ile Cys Ala Asn Phe Cys Ser
 5 10 15

tgt cgg gac act tct gca acc ccg cag agc gca tcc atc aaa gct ttg 274
 Cys Arg Asp Thr Ser Ala Thr Pro Gln Ser Ala Ser Ile Lys Ala Leu
 20 25 30

cgc aac gcc aac ctc agg cga gat gag agc aat cac ctc aca gac ttg 322
 Arg Asn Ala Asn Leu Arg Arg Asp Glu Ser Asn His Leu Thr Asp Leu
 35 40 45

tac cga aga gat gag acc atc cag gtg aaa gga aac ggc tac gtg cag 370
 Tyr Arg Arg Asp Glu Thr Ile Gln Val Lys Gly Asn Gly Tyr Val Gln

50	55	60	65	
agt cct aga ttc ccg aac agc tac ccc agg aac ctg ctc ctg aca tgg				418
Ser Pro Arg Phe Pro Asn Ser Tyr Pro Arg Asn Leu Leu Leu Thr Trp				
	70	75	80	
cgg ctt cac tct cag gag aat aca cgg ata cag cta gtg ttt gac aat				466
Arg Leu His Ser Gln Glu Asn Thr Arg Ile Gln Leu Val Phe Asp Asn				
	85	90	95	
cag ttt gga tta gag gaa gca gaa aat gat atc tgt agg tat gat ttt				514
Gln Phe Gly Leu Glu Glu Ala Glu Asn Asp Ile Cys Arg Tyr Asp Phe				
	100	105	110	
gtg gaa gtt gaa gat ata tcc gaa acc agt acc att att aga gga cga				562
Val Glu Val Glu Asp Ile Ser Glu Thr Ser Thr Ile Ile Arg Gly Arg				
	115	120	125	
tgg tgt gga cac aag gaa gtt cct cca agg ata aaa tca aga acg aac				610
Trp Cys Gly His Lys Glu Val Pro Pro Arg Ile Lys Ser Arg Thr Asn				
	130	135	140	145
caa att aaa atc aca ttc aag tcc gat gac tac ttt gtg gct aaa cct				658
Gln Ile Lys Ile Thr Phe Lys Ser Asp Asp Tyr Phe Val Ala Lys Pro				
	150	155	160	
gga ttc aag att tat tat tct ttg ctg gaa gat ttc caa ccc gca gca				706
Gly Phe Lys Ile Tyr Tyr Ser Leu Leu Glu Asp Phe Gln Pro Ala Ala				
	165	170	175	
gct tca gag acc aac tgg gaa tct gtc aca agc tct att tca ggg gta				754
Ala Ser Glu Thr Asn Trp Glu Ser Val Thr Ser Ser Ile Ser Gly Val				
	180	185	190	
tcc tat aac tct cca tca gta acg gat ccc act ctg att gcg gat gct				802
Ser Tyr Asn Ser Pro Ser Val Thr Asp Pro Thr Leu Ile Ala Asp Ala				
	195	200	205	
ctg gac aaa aaa att gca gaa ttt gat aca gtg gaa gat ctg ctc aag				850
Leu Asp Lys Lys Ile Ala Glu Phe Asp Thr Val Glu Asp Leu Leu Lys				
	210	215	220	225
tac ttc aat cca gag tca tgg caa gaa gat ctt gag aat atg tat ctg				898
Tyr Phe Asn Pro Glu Ser Trp Gln Glu Asp Leu Glu Asn Met Tyr Leu				
	230	235	240	
gac acc cct cgg tat cga ggc agg tca tac cat gac cgg aag tca aaa				946
Asp Thr Pro Arg Tyr Arg Gly Arg Ser Tyr His Asp Arg Lys Ser Lys				

245	250	255	
gtt gac ctg gat agg ctc aat gat gat gcc aag cgt tac agt tgc act			994
Val Asp Leu Asp Arg Leu Asn Asp Asp Ala Lys Arg Tyr Ser Cys Thr			
260	265	270	
ccc agg aat tac tcg gtc aat ata aga gaa gag ctg aag ttg gcc aat			1042
Pro Arg Asn Tyr Ser Val Asn Ile Arg Glu Glu Leu Lys Leu Ala Asn			
275	280	285	
gtg gtc ttc ttt cca cgt tgc ctc ctc gtg cag cgc tgt gga gga aat			1090
Val Val Phe Phe Pro Arg Cys Leu Leu Val Gln Arg Cys Gly Gly Asn			
290	295	300	305
tgt ggc tgt gga act gtc aac tgg agg tcc tgc aca tgc aat tca ggg			1138
Cys Gly Cys Gly Thr Val Asn Trp Arg Ser Cys Thr Cys Asn Ser Gly			
310	315	320	
aaa acc gtg aaa aag tat cat gag gta tta cag ttt gag cct ggc cac			1186
Lys Thr Val Lys Lys Tyr His Glu Val Leu Gln Phe Glu Pro Gly His			
325	330	335	
atc aag agg agg ggt aga gct aag acc atg gct cta gtt gac atc cag			1234
Ile Lys Arg Arg Gly Arg Ala Lys Thr Met Ala Leu Val Asp Ile Gln			
340	345	350	
ttg gat cac cat gaa cga tgc gat tgt atc tgc agc tca aga cca cct			1282
Leu Asp His His Glu Arg Cys Asp Cys Ile Cys Ser Ser Arg Pro Pro			
355	360	365	
cga taa gagaatgtgc acatccttac attaagcctg aaagaacctt tagtttaagg			1338
Arg			
370			
agggtgagat aagagaccct ttctctacca gcaaccaaac ttactactag cctgcaatgc			1398
aatgaacaca agtggttgct gaqtctcagc cttgctttgt taatgccatg gcaagtagaa			1458
agggtatatca tcaacttcta tacctaagaa tataggattg catttaataa tagtgtttga			1518
gggtatatat gcacaaacac acacagaaat atattcatgt ctatgtgtat atagatcaaa			1578
tgtttttttt ggtatatata accaggtaca ccagagctta catatgtttg agttagactc			1638
ttaaaatcct ttgccaaaat aagggatggt caaatatatg aaacatgtct ttagaaaaatt			1698
taggagataa atttattttt aaattttgaa acacaaaaca attttgaatc ttgctctctt			1758

aaagaaagca tcttgatatataaaaaatcaa aagatgaggc tttcttacat atacatctta 1818
 gttgattatt aaaaaaggaa aaagggtttcc agagaaaagg ccaataccta agcatttttt 1878
 ccatgagaag cactgcatac ttacctatgt ggactgtaat aacctgtctc caaaaccatg 1938
 ccataataat ataagtgcct tagaaattaa atcatttgtgt tttttatgca ttttgctgag 1998
 gcatccttat tcatttaaca cctatctcaa aaacttactt agaagggttt ttattatagt 2058
 cctacaaaag acaatgtata agctgtaaca gaattttgaa ttgtttttct ttgcaaaacc 2118
 cctccacaaa agcaaatacct ttcaagaatg gcatgggcat tctgtatgaa cctttccaga 2178
 tgggtgtcag tgaaagatgt gggtagttga gaacttaaaa agtgaacatt gaaacatcga 2238
 cgtaactgga aaccg 2253

<210> 8
 <211> 370
 <212> PRT
 <213> Homo sapiens

<400> 8
 Met His Arg Leu Ile Phe Val Tyr Thr Leu Ile Cys Ala Asn Phe Cys
 1 5 10 15
 Ser Cys Arg Asp Thr Ser Ala Thr Pro Gln Ser Ala Ser Ile Lys Ala
 20 25 30
 Leu Arg Asn Ala Asn Leu Arg Arg Asp Glu Ser Asn His Leu Thr Asp
 35 40 45
 Leu Tyr Arg Arg Asp Glu Thr Ile Gln Val Lys Gly Asn Gly Tyr Val
 50 55 60
 Gln Ser Pro Arg Phe Pro Asn Ser Tyr Pro Arg Asn Leu Leu Leu Thr
 65 70 75 80
 Trp Arg Leu His Ser Gln Glu Asn Thr Arg Ile Gln Leu Val Phe Asp
 85 90 95
 Asn Gln Phe Gly Leu Glu Glu Ala Glu Asn Asp Ile Cys Arg Tyr Asp
 100 105 110
 Phe Val Glu Val Glu Asp Ile Ser Glu Thr Ser Thr Ile Ile Arg Gly
 115 120 125

Arg Trp Cys Gly His Lys Glu Val Pro Pro Arg Ile Lys Ser Arg Thr
 130 135 140
 Asn Gln Ile Lys Ile Thr Phe Lys Ser Asp Asp Tyr Phe Val Ala Lys
 145 150 155 160
 Pro Gly Phe Lys Ile Tyr Tyr Ser Leu Leu Glu Asp Phe Gln Pro Ala
 165 170 175
 Ala Ala Ser Glu Thr Asn Trp Glu Ser Val Thr Ser Ser Ile Ser Gly
 180 185 190
 Val Ser Tyr Asn Ser Pro Ser Val Thr Asp Pro Thr Leu Ile Ala Asp
 195 200 205
 Ala Leu Asp Lys Lys Ile Ala Glu Phe Asp Thr Val Glu Asp Leu Leu
 210 215 220
 Lys Tyr Phe Asn Pro Glu Ser Trp Gln Glu Asp Leu Glu Asn Met Tyr
 225 230 235 240
 Leu Asp Thr Pro Arg Tyr Arg Gly Arg Ser Tyr His Asp Arg Lys Ser
 245 250 255
 Lys Val Asp Leu Asp Arg Leu Asn Asp Asp Ala Lys Arg Tyr Ser Cys
 260 265 270
 Thr Pro Arg Asn Tyr Ser Val Asn Ile Arg Glu Glu Leu Lys Leu Ala
 275 280 285
 Asn Val Val Phe Phe Pro Arg Cys Leu Leu Val Gln Arg Cys Gly Gly
 290 295 300
 Asn Cys Gly Cys Gly Thr Val Asn Trp Arg Ser Cys Thr Cys Asn Ser
 305 310 315 320
 Gly Lys Thr Val Lys Lys Tyr His Glu Val Leu Gln Phe Glu Pro Gly
 325 330 335
 His Ile Lys Arg Arg Gly Arg Ala Lys Thr Met Ala Leu Val Asp Ile
 340 345 350
 Gln Leu Asp His His Glu Arg Cys Asp Cys Ile Cys Ser Ser Arg Pro
 355 360 365
 Pro Arg
 370

<210> 9
 <211> 4
 <212> PRT
 <213> Homo sapiens

<400> 9
 Arg Lys Ser Lys
 1

<210> 10
 <211> 192
 <212> PRT
 <213> Homo sapiens

<400> 10
 Met Asn Phe Leu Leu Ser Trp Val His Trp Ser Leu Ala Leu Leu Leu
 1 5 10 15
 Tyr Leu His His Ala Lys Trp Ser Gln Ala Ala Pro Met Ala Glu Gly
 20 25 30
 Gly Gly Gln Asn His His Glu Val Val Lys Phe Met Asp Val Tyr Gln
 35 40 45
 Arg Ser Tyr Cys His Pro Ile Glu Thr Leu Val Asp Ile Phe Gln Glu
 50 55 60
 Tyr Pro Asp Glu Ile Glu Tyr Ile Phe Lys Pro Ser Cys Val Pro Leu
 65 70 75 80
 Met Arg Cys Gly Gly Cys Cys Asn Asp Glu Gly Leu Glu Cys Val Pro
 85 90 95
 Thr Glu Glu Ser Asn Ile Thr Met Gln Ile Met Arg Ile Lys Pro His
 100 105 110
 Gln Gly Gln His Ile Gly Glu Met Ser Phe Leu Gln His Asn Lys Cys
 115 120 125
 Glu Cys Arg Pro Lys Lys Asp Arg Ala Arg Gln Glu Asn Pro Cys Gly
 130 135 140
 Pro Cys Ser Ser Glu Arg Arg Lys His Leu Phe Val Gln Asp Pro Gln
 145 150 155 160

Thr Cys Lys Cys Ser Cys Lys Asn Thr Asp Ser Arg Cys Lys Ala Arg
 165 170 175

Gln Leu Glu Leu Asn Glu Arg Thr Cys Arg Cys Asp Lys Pro Arg Arg
 180 185 190

<210> 11

<211> 170

<212> PRT

<213> Homo sapiens

<400> 11

Met Pro Val Met Arg Leu Phe Pro Cys Phe Leu Gln Leu Leu Ala Gly
 1 5 10 15

Leu Ala Leu Pro Ala Val Pro Pro Gln Gln Trp Ala Leu Ser Ala Gly
 20 25 30

Asn Gly Ser Ser Glu Val Glu Val Val Pro Phe Gln Glu Val Trp Gly
 35 40 45

Arg Ser Tyr Cys Arg Ala Leu Glu Arg Leu Val Asp Val Val Ser Glu
 50 55 60

Tyr Pro Ser Glu Val Glu His Met Phe Ser Pro Ser Cys Val Ser Leu
 65 70 75 80

Leu Arg Cys Thr Gly Cys Cys Gly Asp Glu Asp Leu His Cys Val Pro
 85 90 95

Val Glu Thr Ala Asn Val Thr Met Gln Leu Leu Lys Ile Arg Ser Gly
 100 105 110

Asp Arg Pro Ser Tyr Val Glu Leu Thr Phe Ser Gln His Val Arg Cys
 115 120 125

Glu Cys Arg Pro Leu Arg Glu Lys Met Lys Pro Glu Arg Arg Arg Pro
 130 135 140

Lys Gly Arg Gly Lys Arg Arg Arg Glu Asn Gln Arg Pro Thr Asp Cys
 145 150 155 160

His Leu Cys Gly Asp Ala Val Pro Arg Arg
 165 170

<210> 12
 <211> 188
 <212> PRT
 <213> Homo sapiens

<400> 12
 Met Ser Pro Leu Leu Arg Arg Leu Leu Leu Ala Ala Leu Leu Gln Leu
 1 5 10 15
 Ala Pro Ala Gln Ala Pro Val Ser Gln Pro Asp Ala Pro Gly His Gln
 20 25 30
 Arg Lys Val Val Ser Trp Ile Asp Val Tyr Thr Arg Ala Thr Cys Gln
 35 40 45
 Pro Arg Glu Val Val Val Pro Leu Thr Val Glu Leu Met Gly Thr Val
 50 55 60
 Ala Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly
 65 70 75 80
 Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln
 85 90 95
 Val Arg Met Gln Ile Leu Met Ile Arg Tyr Pro Ser Ser Gln Leu Gly
 100 105 110
 Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys
 115 120 125
 Lys Asp Ser Ala Val Lys Pro Asp Ser Pro Arg Pro Leu Cys Pro Arg
 130 135 140
 Cys Thr Gln His His Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg
 145 150 155 160
 Cys Arg Arg Arg Ser Phe Leu Arg Cys Gln Gly Arg Gly Leu Glu Leu
 165 170 175
 Asn Pro Asp Thr Cys Arg Cys Arg Lys Leu Arg Arg
 180 185

<210> 13
 <211> 364
 <212> PRT

<213> Homo sapiens

<400> 13

Met Glu Thr Leu Tyr Ser Leu Glu Leu Glu Val Ala Leu Gly Leu Tyr
 1 5 10 15

Ile Leu Glu Leu Glu Val Ala Leu Ala Leu Ala Val Ala Leu Cys Tyr
 20 25 30

Ser Leu Glu His Ile Ser Gly Leu Asn Thr Tyr Arg Leu Glu Leu Glu
 35 40 45

Ala Ser Asn Ala Leu Ala Ala Ser Pro Ser Glu Arg Ala Ser Asn Thr
 50 55 60

His Arg Leu Tyr Ser Gly Leu Tyr Thr Arg Pro Ser Glu Arg Gly Leu
 65 70 75 80

Val Ala Leu Leu Glu Leu Tyr Ser Gly Leu Tyr Ser Glu Arg Gly Leu
 85 90 95

Cys Tyr Ser Leu Tyr Ser Pro Arg Ala Arg Gly Pro Arg Ile Leu Glu
 100 105 110

Val Ala Leu Val Ala Leu Pro Arg Val Ala Leu Ser Glu Arg Gly Leu
 115 120 125

Thr His Arg His Ile Ser Pro Arg Gly Leu Leu Glu Thr His Arg Ser
 130 135 140

Glu Arg Gly Leu Asn Ala Arg Gly Pro His Glu Ala Ser Asn Pro Arg
 145 150 155 160

Pro Arg Cys Tyr Ser Val Ala Leu Thr His Arg Leu Glu Met Glu Thr
 165 170 175

Ala Arg Gly Cys Tyr Ser Gly Leu Tyr Gly Leu Tyr Cys Tyr Ser Cys
 180 185 190

Tyr Ser Ala Ser Asn Ala Ser Pro Gly Leu Ser Glu Arg Leu Glu Gly
 195 200 205

Leu Cys Tyr Ser Val Ala Leu Pro Arg Thr His Arg Gly Leu Gly Leu
 210 215 220

Val Ala Leu Ala Ser Asn Val Ala Leu Ser Glu Arg Met Glu Thr Gly
 225 230 235 240

Leu Leu Glu Leu Glu Gly Leu Tyr Ala Leu Ala Ser Glu Arg Gly Leu
245 250 255

Tyr Ser Glu Arg Gly Leu Tyr Ser Glu Arg Ala Ser Asn Gly Leu Tyr
260 265 270

Met Glu Thr Gly Leu Asn Ala Arg Gly Leu Glu Ser Glu Arg Pro His
275 280 285

Glu Val Ala Leu Gly Leu His Ile Ser Leu Tyr Ser Leu Tyr Ser Cys
290 295 300

Tyr Ser Ala Ser Pro Cys Tyr Ser Ala Arg Gly Pro Arg Ala Arg Gly
305 310 315 320

Pro His Glu Thr His Arg Thr His Arg Thr His Arg Pro Arg Pro Arg
325 330 335

Thr His Arg Thr His Arg Thr His Arg Ala Arg Gly Pro Arg Pro Arg
340 345 350

Ala Arg Gly Ala Arg Gly Ala Arg Gly Ala Arg Gly
355 360

<210> 14

<211> 419

<212> PRT

<213> Homo sapiens

<400> 14

Met His Leu Leu Gly Phe Phe Ser Val Ala Cys Ser Leu Leu Ala Ala
1 5 10 15

Ala Leu Leu Pro Gly Pro Arg Glu Ala Pro Ala Ala Ala Ala Phe
20 25 30

Glu Ser Gly Leu Asp Leu Ser Asp Ala Glu Pro Asp Ala Gly Glu Ala
35 40 45

Thr Ala Tyr Ala Ser Lys Asp Leu Glu Glu Gln Leu Arg Ser Val Ser
50 55 60

Ser Val Asp Glu Leu Met Thr Val Leu Tyr Pro Glu Tyr Trp Lys Met
65 70 75 80

Tyr Lys Cys Gln Leu Arg Lys Gly Gly Trp Gln His Asn Arg Glu Gln
85 90 95

Ala Asn Leu Asn Ser Arg Thr Glu Glu Thr Ile Lys Phe Ala Ala Ala
 100 105 110
 His Tyr Asn Thr Glu Ile Leu Lys Ser Ile Asp Asn Glu Trp Arg Lys
 115 120 125
 Thr Gln Cys Met Pro Arg Glu Val Cys Ile Asp Val Gly Lys Glu Phe
 130 135 140
 Gly Val Ala Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Ser Val Tyr
 145 150 155 160
 Arg Cys Gly Gly Cys Cys Asn Ser Glu Gly Leu Gln Cys Met Asn Thr
 165 170 175
 Ser Thr Ser Tyr Leu Ser Lys Thr Leu Phe Glu Ile Thr Val Pro Leu
 180 185 190
 Ser Gln Gly Pro Lys Pro Val Thr Ile Ser Phe Ala Asn His Thr Ser
 195 200 205
 Cys Arg Cys Met Ser Lys Leu Asp Val Tyr Arg Gln Val His Ser Ile
 210 215 220
 Ile Arg Arg Ser Leu Pro Ala Thr Leu Pro Gln Cys Gln Ala Ala Asn
 225 230 235 240
 Lys Thr Cys Pro Thr Asn Tyr Met Trp Asn Asn His Ile Cys Arg Cys
 245 250 255
 Leu Ala Gln Glu Asp Phe Met Phe Ser Ser Asp Ala Gly Asp Asp Ser
 260 265 270
 Thr Asp Gly Phe His Asp Ile Cys Gly Pro Asn Lys Glu Leu Asp Glu
 275 280 285
 Glu Thr Cys Gln Cys Val Cys Arg Ala Gly Leu Arg Pro Ala Ser Cys
 290 295 300
 Gly Pro His Lys Glu Leu Asp Arg Asn Ser Cys Gln Cys Val Cys Lys
 305 310 315 320
 Asn Lys Leu Phe Pro Ser Gln Cys Gly Ala Asn Arg Glu Phe Asp Glu
 325 330 335
 Asn Thr Cys Gln Cys Val Cys Lys Arg Thr Cys Pro Arg Asn Gln Pro
 340 345 350

Leu Asn Pro Gly Lys Cys Ala Cys Glu Cys Thr Glu Ser Pro Gln Lys
355 360 365

Cys Leu Leu Lys Gly Lys Lys Phe His His Gln Thr Cys Ser Cys Tyr
370 375 380

Arg Arg Pro Cys Thr Asn Arg Gln Lys Ala Cys Glu Pro Gly Phe Ser
385 390 395 400

Tyr Ser Glu Glu Val Cys Arg Cys Val Pro Ser Tyr Trp Lys Arg Pro
405 410 415

Gln Met Ser

<210> 15

<211> 358

<212> PRT

<213> Homo sapiens

<400> 15

Met Tyr Gly Glu Trp Gly Met Gly Asn Ile Leu Met Met Phe His Val
1 5 10 15

Tyr Leu Val Gln Gly Phe Arg Ser Glu His Gly Pro Val Lys Asp Phe
20 25 30

Ser Phe Glu Arg Ser Ser Arg Ser Met Leu Glu Arg Ser Glu Gln Gln
35 40 45

Ile Arg Ala Ala Ser Ser Leu Glu Glu Leu Leu Gln Ile Ala His Ser
50 55 60

Glu Asp Trp Lys Leu Trp Arg Cys Arg Leu Lys Leu Lys Ser Leu Ala
65 70 75 80

Ser Met Asp Ser Arg Ser Ala Ser His Arg Ser Thr Arg Phe Ala Ala
85 90 95

Thr Phe Tyr Asp Thr Glu Thr Leu Lys Val Ile Asp Glu Glu Trp Gln
100 105 110

Arg Thr Gln Cys Ser Pro Arg Glu Thr Cys Val Glu Val Ala Ser Glu
115 120 125

Leu Gly Lys Thr Thr Asn Thr Phe Phe Lys Pro Pro Cys Val Asn Val

130	135	140																	
Phe	Arg	Cys	Gly	Gly	Cys	Cys	Asn	Glu	Glu	Gly	Val	Met	Cys	Met	Asn				
145					150					155					160				
Thr	Ser	Thr	Ser	Tyr	Ile	Ser	Lys	Gln	Leu	Phe	Glu	Ile	Ser	Val	Pro				
				165					170						175				
Leu	Thr	Ser	Val	Pro	Glu	Leu	Val	Pro	Val	Lys	Ile	Ala	Asn	His	Thr				
			180					185						190					
Gly	Cys	Lys	Cys	Leu	Pro	Thr	Gly	Pro	Arg	His	Pro	Tyr	Ser	Ile	Ile				
		195					200						205						
Arg	Arg	Ser	Ile	Gln	Thr	Pro	Glu	Glu	Asp	Glu	Cys	Pro	His	Ser	Lys				
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Lys	Leu	Cys	Pro	Ile	Asp	Met	Leu	Trp	Asp	Asn	Thr	Lys	Cys	Lys	Cys				
225					230					235					240				
Val	Leu	Gln	Asp	Glu	Thr	Pro	Leu	Pro	Gly	Thr	Glu	Asp	His	Ser	Tyr				
			245						250						255				
Leu	Gln	Glu	Pro	Thr	Leu	Cys	Gly	Pro	His	Met	Thr	Phe	Asp	Glu	Asp				
			260					265						270					
Arg	Cys	Glu	Cys	Val	Cys	Lys	Ala	Pro	Cys	Pro	Gly	Asp	Leu	Ile	Gln				
		275					280						285						
His	Pro	Glu	Asn	Cys	Ser	Cys	Phe	Glu	Cys	Lys	Glu	Ser	Leu	Glu	Ser				
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Cys	Cys	Gln	Lys	His	Lys	Ile	Phe	His	Pro	Asp	Thr	Cys	Ser	Cys	Glu				
305					310					315					320				
Asp	Arg	Cys	Pro	Phe	His	Thr	Arg	Thr	Cys	Ala	Ser	Arg	Lys	Pro	Ala				
			325						330					335					
Cys	Gly	Lys	His	Trp	Arg	Phe	Pro	Lys	Glu	Thr	Arg	Ala	Gln	Gly	Leu				
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Tyr	Ser	Gln	Glu	Asn	Pro														
			355																

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<211> 211

<212> PRT

<213> Homo sapiens

<400> 16

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 20 25 30

Leu Ala Arg Ser Gln Ile His Ser Ile Arg Asp Leu Gln Arg Leu Leu
 35 40 45

Glu Ile Asp Ser Val Gly Ser Glu Asp Ser Leu Asp Thr Ser Leu Arg
 50 55 60

Ala His Gly Val His Ala Thr Lys His Val Pro Glu Lys Arg Pro Leu
 65 70 75 80

Pro Ile Arg Arg Lys Arg Ser Ile Glu Glu Ala Val Pro Ala Val Cys
 85 90 95

Lys Thr Arg Thr Val Ile Tyr Glu Ile Pro Arg Ser Gln Val Asp Pro
 100 105 110

Thr Ser Ala Asn Phe Leu Ile Trp Pro Pro Cys Val Glu Val Lys Arg
 115 120 125

Cys Thr Gly Cys Cys Asn Thr Ser Ser Val Lys Cys Gln Pro Ser Arg
 130 135 140

Val His His Arg Ser Val Lys Val Ala Lys Val Glu Tyr Val Arg Lys
 145 150 155 160

Lys Pro Lys Leu Lys Glu Val Gln Val Arg Leu Glu Glu His Leu Glu
 165 170 175

Cys Ala Cys Ala Thr Thr Ser Leu Asn Pro Asp Tyr Arg Glu Glu Asp
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Lys Pro Thr
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<400> 17

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Thr Arg Ser His Ser Gly Gly Glu Leu Glu Ser Leu Ala Arg Gly Arg
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Arg Ser Leu Gly Ser Leu Thr Ile Ala Glu Pro Ala Met Ile Ala Glu
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          100            105            110

Arg Thr Asn Ala Asn Phe Leu Val Trp Pro Pro Cys Val Glu Val Gln
          115            120            125

Arg Cys Ser Gly Cys Cys Asn Asn Arg Asn Val Gln Cys Arg Pro Thr
          130            135            140

Gln Val Gln Leu Arg Pro Val Gln Val Arg Lys Ile Glu Ile Val Arg
          145            150            155            160

Lys Lys Pro Ile Phe Lys Lys Ala Thr Val Thr Leu Glu Asp His Leu
          165            170            175

Ala Cys Lys Cys Glu Thr Val Ala Ala Ala Arg Pro Val Thr Arg Ser
          180            185            190

Pro Gly Gly Ser Gln Glu Gln Arg Ala Lys Thr Pro Gln Thr Arg Val
          195            200            205

Thr Ile Arg Thr Val Arg Val Arg Arg Pro Pro Lys Gly Lys His Arg
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Lys Phe Lys His Thr His Asp Lys Thr Ala Leu Lys Glu Thr Leu Gly
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<211> 121

<212> PRT

<213> Homo sapiens

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 20 25 30

Arg Glu Glu Leu Lys Leu Ala Asn Val Val Phe Phe Pro Arg Cys Leu
 35 40 45

Leu Val Gln Arg Cys Gly Gly Asn Cys Gly Cys Gly Thr Val Lys Leu
 50 55 60

Glu Ser Cys Thr Cys Asn Ser Gly Lys Thr Val Lys Lys Tyr His Glu
 65 70 75 80

Val Leu Gln Phe Glu Pro Gly His Ile Lys Arg Arg Gly Arg Ala Lys
 85 90 95

Thr Met Ala Leu Val Asp Ile Gln Leu Asp His His Glu Arg Cys Asp
 100 105 110

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His Ser Gln Glu Asn Thr Arg Ile Gln Leu Val Phe Asp Asn Gln Phe
 35 40 45
 Gly Leu Glu Glu Ala Glu Asn Asp Ile Cys Arg Tyr Asp Phe Val Glu
 50 55 60
 Val Glu Asp Ile Ser Glu Thr Ser Thr Ile Ile Arg Gly Arg Trp Cys
 65 70 75 80
 Gly His Lys Glu Val Pro Pro Arg Ile Lys Ser Arg Thr Asn Gln Ile
 85 90 95
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 35 40 45
 Tyr Arg Ser Arg Leu Cys Trp Tyr Asp Tyr Val Glu Val Arg Asp Gly
 50 55 60
 Phe Trp Arg Lys Ala Pro Leu Arg Gly Arg Phe Cys Gly Ser Lys Leu
 65 70 75 80
 Pro Glu Pro Ile Val Ser Thr Asp Ser Arg Leu Trp Val Glu Phe Arg
 85 90 95
 Ser Ser Ser Asn Trp Val Gly Lys Gly Phe Phe Ala Val Tyr Glu Ala
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 35 40 45
 Glu Arg Met Asp Ser Cys Ala Tyr Asp Tyr Leu Glu Val Arg Asp Gly
 50 55 60
 His Ser Glu Ser Ser Thr Leu Ile Gly Arg Tyr Cys Gly Tyr Glu Lys
 65 70 75 80
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 20 25 30
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 35 40 45

Glu Gly Asn Asp Val Cys Lys Tyr Asp Phe Val Glu Val Arg Ser Gly
 50 55 60

Leu Thr Ala Asp Ser Lys Leu His Gly Lys Phe Cys Gly Ser Glu Lys
 65 70 75 80

Pro Glu Val Ile Thr Ser Gln Tyr Asn Asn Met Arg Val Glu Pro Lys
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<213> Homo sapiens

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 20 25 30

Ala Pro Asp Pro Tyr Gln Arg Ile Met Ile Asn Phe Asn Pro His Phe
 35 40 45

Asp Leu Glu Asp Arg Asp Cys Lys Tyr Asp Tyr Val Glu Val Phe Asp
 50 55 60

Gly Glu Asn Glu Asn Gly His Phe Arg Gly Lys Phe Cys Gly Lys Ile
 65 70 75 80

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 85 90 95

Val Ser Asp Tyr Glu Thr His Gly Ala Gly Phe Ser Ile Arg Tyr Glu
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 20 25 30

Ala Pro Lys Met Ser Glu Ile Ile Leu Glu Phe Glu Ser Phe Asp Leu
 35 40 45

Glu Pro Asp Ser Asn Pro Pro Gly Gly Met Phe Cys Arg Tyr Asp Arg
 50 55 60

Leu Glu Ile Trp Asp Gly Phe Pro Asp Val Gly Pro His Ile Gly Arg
 65 70 75 80

Tyr Cys Gly Gln Lys Thr Pro Gly Arg Ile Arg Ser Ser Ser Gly Ile
 85 90 95

Leu Ser Met Val Phe Tyr Thr Asp Ser Ala Ile Ala Lys Glu Gly Phe
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Ser Ala Asn Tyr Ser Val Leu
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22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/26462

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C07K 14/49, 14/475; C12N 15/00
US CL : 435/69.1; 536/23.5; 530/350

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. :

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Genbank, EST, Swissprot, Medline, USPatfull
search terms: PDGF-D, VEGF, CUB

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5,607,918 A (ERIKSSON et al) 04 March 1997(04.03.97), col.13-14, SEQ ID No:2 at position 39-51.	10, 30
A	WO 96/27007 A1 (AMRAD OPERATIONS PTY. LTD.) 06 September 1996(06.09.96), see page 41, SEQ ID No:4 at position 70-82.	10, 30
A	Database GENCORE on EST, AN AA488780, NCI-CGAP, 'National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index.' Gene Sequence, 15 August 1997.	1-40, 50, 52-60, 66,67
A	Database GENCORE on EST, AN AA488996, NCI-CGAP, 'National Cancer Institute, Cancer1 Genome Anatomy Project (CGAP), tumor Gene Index.' Gene Sequence, 15 August 1997.	1-40, 50, 52-60, 66, 67

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*A* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

23 MARCH 2000

Date of mailing of the international search report

25 APR 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

ELIANE LAZAR-WESLEY

Telephone No. (703) 308-0196

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/26462

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of documents, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database GENCORE on EST, AN AA736766, NCI-CGAP, 'National Cancer Institute, Cancer Genome Anatomy Project (CGAP), Tumor Gene Index.' Gene Sequence, 23 January 1998.	1-40, 50, 52-60, 66, 67
A	Database GENCORE on EST, AN AQ041639, ADAMS et al. 'Use of a random BAC End Sequence Database for Sequence-Ready Map Building (1998).' Gene Sequence, 14 July 1998.	1-40, 50, 52-60, 66, 67

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/26462

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-40, 50, 52-60, 66, 67
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest



The additional search fees were accompanied by the applicant's protest.



No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US99/26462

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING

This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-40, 50, 55-60, and 66, drawn to nucleic acid molecules having SEQ ID No:3, 5 and 7, vectors, host cells, proteins having SEQ ID No:4, 6 and 8, and method of making a polypeptide.

Group II, claims 41-42, drawn to a method of amplifying a polynucleotide.

Group III, claims 43-49, drawn to antibodies.

Group IV, claim 51, drawn to a method of stimulating growth.

Group V, claims 52-54 and 67, drawn to a method of producing an activated truncated form of PDGF-D.

Group VI, claim 61, drawn to a method of inducing PDGF beta receptor activation.

Group VII, claim 62, drawn to method of inhibiting tumor growth.

Group VIII, claim 63, drawn to a method of identifying types of tumors.

Group IX, claims 64-65, drawn to method of identifying a PDGF-D antagonist.

Group X, claim 68, drawn to a method of inhibiting tissue remodeling.

The inventions listed as Groups I-X do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Pursuant to 37 C.F.R.(d), the ISA/US considers that where multiple products and processes are claimed, the main invention shall consist of the first invention of the category first mentioned in the claims and the first recited invention of each of the other categories related thereto. Accordingly, the main invention (Group I) comprises the first recited product, a polynucleotide of SEQ ID No: 3,5,7 encoding hPDGF-D, a vector, a host cell, a method of producing a hPDGF-D protein of SEQ ID No:4, 6 and 8 and hPDGF-D protein of SEQ ID No:4,6 and 8. Note that there is no method of making the polynucleotide. Further, pursuant to 37 C.F.R. 1.45(d), the ISA/US considers that any feature which the subsequently products of Group III and additional methods of Groups II and IV-X share with the main invention does not constitute a special technical feature within the meaning of PCT Rule 13.2 and that each of such products and methods accordingly defines a separate invention.